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9093

Dynamics of Fibrinolysin-Production by Streptococci.*

R. R. MADISON AND JEANNETTE D. TARANIK. (Introduced by W. H. Manwaring.)

From the Laboratory of Bacteriology and Experimental Pathology, Stanford University, California.

To estimate the amount (or potency) of the fibrinolytic "enzyme" formed or secreted by *Streptococcus hemolyticus,* the following

* Work supported in part by the Eli Lilly and Co. Streptococcus Research Fellowship of Stanford University and in part by the Rockefeller Fluid Research Fund of Stanford Medical School.

titrative method has been adopted by our laboratory. Standard solutions of fibrinogen and thrombin are prepared from freshly drawn human blood by the method of Tillett and Garner.[1] Serial dilutions (1:2) are made of the broth culture to be tested. To 0.5 cc. of each dilution there is added 1 cc. of the standard solution of fibrinogen. Coagulation is then brought about by the addition of 0.1 cc. of standard solution of thrombin, the fibrinous clot usually forming within 30 seconds, after which each tube is placed in a thermostatic waterbath (37°C.). Readings are usually made at the end of 15 minutes, 30 minutes, 60 minutes and 2 hours. The highest serial dilution of the broth culture causing complete liquefaction of the clot by the end of 2 hours' incubation is assumed to contain one arbitrary fibrinolytic unit. From this dilution the number of lytic units per cc. of broth culture is readily calculated.

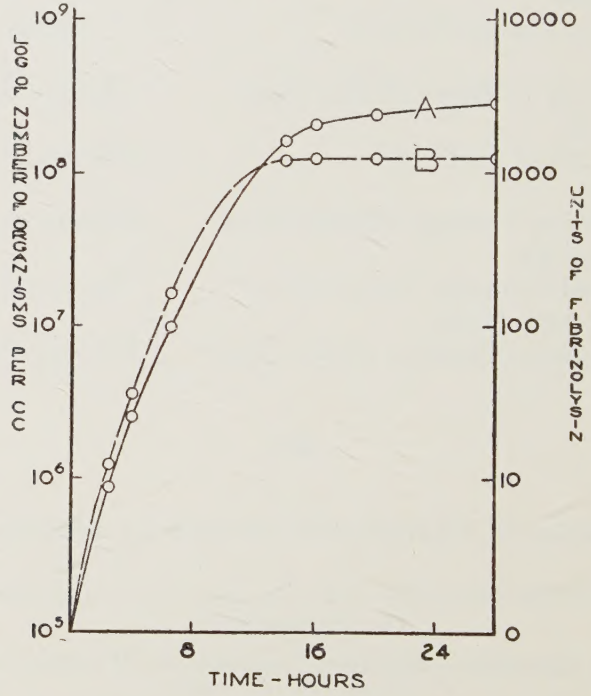

FIG. 1.

Relation of fibrinolytic titer to proliferation-rate. 100 cc. veal-infusion dextrose-broth plus 0.1 cc. 24-hour broth culture of *S. hemolyticus;* incubated at 37°C with constant stirring.

A, Increase in total population per cc. as determined by Petroff-Hausser counting chamber.

B, Parallel changes in fibrinolytic titer per 0.5 cc., plotted as a logarithmic function.

[1] Tillett, W. S., and Garner, R. L., *J. Exp. Med.*, 1933, **58**, 485.

Using this method, the quantitative laws of fibrinolysin-production have been determined for numerous strains of *S. hemolyticus.* Typical data thus obtained are recorded in Fig. 1.

From this curve it is evident that the rate of fibrinolysin-production and the rate of test-tube proliferation of streptococci are parallel during the logarithmic phase of population-increase, an apparent quantitative linkage between enzyme secretion and cell-division.

This apparent linkage, however, is not operative beyond the logarithmic phase of population-increase. While with certain strains the fibrinolytic titer remains constant or even increases slightly during the subsequent static phase of test-tube growth, a fairly rapid destruction (or inactivation) of the lytic factor takes place in most older cultures. Three typical sets of data are recorded in Fig. 2.

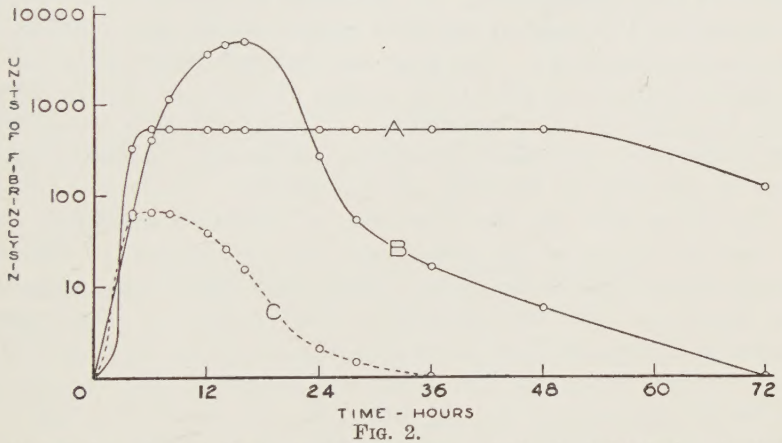

FIG. 2.

Destruction of fibrinolysin in older cultures. 100 cc. veal-infusion dextrose-broth plus 0.1 cc. 24-hour broth culture of *S. hemolyticus;* incubated at 37.5°C with constant stirring.

A, Changes in fibrinolytic titer per 0.5 cc. in Streptococcus Strain No. 211.
B, Changes in fibrinolytic titer per 0.5 cc. in Streptococcus Strain No. 291
C, Changes in fibrinolytic titer per 0.5 cc. in Streptococcus Strain No. 189

From these data it is evident that a routine clinical test of 18- to 24-hour broth cultures of *S. hemolyticus* may lead to erroneous conclusions as to their fibrinolytic (or invasive) properties. A routine test of younger (*e. g.,* 12-hour) broth cultures would presumably lead to greater clinical accuracy.

9094

Vibratory Sensibility: Influence of Height and Surface Area.*

KENDALL B. CORBIN AND HENRY W. NEWMAN.

From the Department of Anatomy and the Department of Medicine, Stanford University School of Medicine.

An apparatus for the quantitative determination of vibratory sensibility has been described by Newman and Corbin,[1] who determined vibratory acuity of normal individuals and found a progressive increase in threshold with age. An apparent tendency for tall individuals to exhibit higher thresholds was noticed during the course of that investigation. To determine this point, the vibratory thresholds of 139 males in the third decade of life were obtained as previously described.[1] The data so obtained were subjected to statistical analysis, in order to determine whether any relation might exist between vibratory sensibility and weight, height, or surface area. Surface area was computed by means of a DuBois surface chart as prepared by Boothby and Sandiford.

The frequencies for the thresholds of vibratory sensibility over the cranial margin of the patellae and the frequencies for different heights have been distributed in Contingency Table I, and those for the thresholds over the malleoli and for height, in Contingency Table II. Three numbers are shown in each cell in the tables. The first number represents the observed frequency, the second the theoretical frequency, and the third the chi square for that cell. Tables III and IV similarly represent the relation between vibratory sensibility threshold frequencies and surface area frequencies. The data have been analyzed by the chi square distribution method as used by Fisher.[2] The total chi square for each table, when referred to a Table of Goodness of Fit as given by Fisher, indicated that the probability that the discrepancy between the observed and the theoretical figures was due to chance was less than one in one hundred. Examination of Tables I to IV further reveals that the greatest discrepancies occur in those cells containing the extremes of the variables studied, and indicates a positive correlation between stature

* This study was supported in part by the Rockefeller Foundation Grant for Fluid Research in the Medical Sciences at Stanford University.

1 Newman, H. W., and Corbin, K. B., PROC. SOC. EXP. BIOL. AND MED. In publication.

2 Fisher, R. A., Chap. IV, *Statistical Methods for Research Workers*, Oliver and Boyd, Edinburgh, 1932.

and threshold of vibratory acuity. The chi square test showed a less significant relationship between vibratory acuity and weight.

TABLE I.
Distribution of 139 Males, 20-29 Years of Age.

Height, inches		Vibratory Threshold over Patellae					
		0.0-2.0	2.1-2.3	2.4-2.6	2.7-3.0	3.1 plus	Totals f_o
0.0-69.9	f_o	15	13	7	2	3	40
	f	12.66	7.19	6.91	4.89	8.35	
	x_2	0.43	4.69	0.00	1.71	3.43	
70.0-71.9	f_o	18	7	10	7	8	50
	f	15.83	8.99	8.63	6.17	10.43	
	x_2	0.30	0.44	0.22	0.11	0.57	
72.0 plus	f_o	11	5	7	8	18	49
	f	15.51	8.81	8.46	5.99	10.22	
	x_2	1.31	1.65	0.25	0.67	5.92	
Totals f_o		44	25	24	17	29	139

f_o = observed frequency, f = theoretical frequency, n = no. of degrees of freedom = 8, total x_2 = 21.70.

TABLE II.
Distribution of 139 Males, 20-29 Years of Age.

Height, inches		Vibratory Threshold over Malleoli				
		0.0-0.3	0.4-0.6	0.7-1.0	1.1 plus	Totals f_o
0.0-69.9	f_o	16	9	10	6	41
	f	8.26	10.03	10.32	12.39	
	x_2	7.25	0.11	0.01	3.30	
70.0-71.9	f_o	8	13	14	14	49
	f	9.87	11.99	12.34	14.805	
	x_2	0.35	0.09	0.22	0.04	
72.0 plus	f_o	4	12	11	22	49
	f	9.87	11.99	12.34	14.81	
	x_2	3.49	0.00	0.15	3.49	
Totals f_o		28	34	35	42	139

f_o = observed frequency, f = theoretical frequency, n = no. of degrees of freedom = 6, total x_2 = 18.50.

The evidence presented shows that individuals of greater height and surface area tend to have a higher vibratory threshold. That this increase in threshold could be due to other factors seems un-

TABLE III.
Distribution of 139 Males, 20-29 Years of Age.

Surface Area Square Meters		Vibratory Threshold over Patellae				
		0.0-2.0	2.1-2.3	2.4-2.6	2.7 plus	Totals f_o
0.0-1.85	f_o	22	14	9	9	54
	f	17.48	9.32	9.71	17.48	
	x_2	1.17	2.35	.05	4.11	
1.86-1.95	f_o	13	6	10	10	39
	f	12.63	6.73	7.01	12.63	
	x_2	0.01	0.08	1.28	.55	
1.96 plus	f_o	10	4	6	26	46
	f	14.89	7.94	8.27	14.89	
	x_2	1.61	1.96	0.62	8.29	
Totals f_o		45	24	25	45	139

f_o = observed frequency, f = theoretical frequency, n = no. of degrees of freedom = 6, total x_2 = 22.08.

TABLE IV.
Distribution of 139 Males, 20-29 Years of Age.

Surface Area Square Meters		Vibratory Threshold over Malleoli				
		0.0-0.3	0.4-0.6	0.7-1.0	1.1 plus	Totals f_o
0.0-1.85	f_o	20	12	11	10	53
	f	11.06	12.58	12.96	16.40	
	x_2	7.23	0.03	.29	2.50	
1.86-1.95	f_o	5	10	11	13	39
	f	8.14	9.26	9.54	12.06	
	x_2	1.21	0.06	0.22	0.07	
1.96 plus	f_o	4	11	12	20	47
	f	9.81	11.16	11.50	14.54	
	x_2	3.44	0.00	0.02	2.05	
Totals f_o		29	33	34	43	139

f_o = observed frequency, f = theoretical frequency, n = no. of degrees of freedom = 6, total x_2 = 17.12.

likely. Since the subjects were within the third decade of life, a decrease in vibratory acuity because of age would not be expected.[1, 3] Moreover, since this group consisted of medical students, it was unusually homogeneous and therefore reacted in a consistent manner to the examination.

[3] Pearson, G. H. J., *Arch. Neurol. and Psychiat.*, 1928, **20**, 482.

The reduced acuity in individuals of greater body surface may be due to their possession of fewer sensory receptors per unit area. Further investigation is needed to establish this point.

Individuals of greater height and greater surface area appear to have a higher threshold of vibratory sensibility.

9095 P

Propagation of Variola Virus in the Developing Egg.

A. S. LAZARUS,* B. EDDIE AND K. F. MEYER.

From the George Williams Hooper Foundation, University of California, and the San Francisco Hospital, Department of Public Health, San Francisco.

Since the early reports of Goodpasture and his coworkers[1, 2, 3] the chorio-allantoic membrane of the developing chick has been used for the propagation of a number of filterable viruses. This paper reports the propagation of the virus of variola major isolated directly from the pustular content of an active case. It is now in the forty-fifth consecutive passage on the chorio-allantoic membrane of the developing egg. The propagation of alastrim[4] has been attempted only after 2 passages through Macacus rhesus monkeys.

The patient, an unvaccinated white woman aged 32, developed a typical case of confluent smallpox shortly after a visit to Mexico City. The material for propagation was removed from lesions on the lower leg and soles of the feet on the seventeenth day after the onset of the disease. The abdominal and back lesions were starting to peel at this time. The material was obtained from 4 or 5 vesicles by means of a 1 cc. tuberculin syringe and consisted of 0.3 cc. of slightly turbid fluid and some swabs moistened with vesicle fluid. Growth was first obtained in one egg inoculated with 0.1 cc. of the vesicle fluid. Four days after inoculation, about 20 discrete yellowish white lesions were observed on the membrane. Impression smears showed typical Paschen bodies with Morosow's stain.[5]

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* Edith Claypole Memorial Research Fellow in Pathology, 1936-37.

1 Woodruff, A. M., and Goodpasture, E. W., *Am. J. Path.*, 1931, **7**, 209.

2 Goodpasture, E. W., Woodruff, A. M., and Buddingh, G. J., *Science*, 1931, **74**, 371.

3 Goodpasture, E. W., Woodruff, A. M., and Buddingh, G. J., *Am. J. Path.*, 1932, **8**, 271.

4 Torres, C. M., and Teixeira, J. deC., *Compt. rend. Soc. de biol.*, 1935, **118**, 1023.

5 Morosow, M. A., *Zentralbl. f. Bakt. Abt.* 1, 1926, **100**, 385.

The same findings were noted in the second egg, 5 days after inoculation with 0.1 cc. of a saline suspension of the swab material. The usual shell-flap method of Goodpasture was used throughout. Passage was made every 4 to 5 days by transferring a piece of membrane about 1 mm. in diameter, containing 2 or more lesions, to the exposed membrane of a 11-13 day egg. Six to 8 eggs were inoculated at each passage. No bacterial contaminations were encountered in 45 passages, and no dead embryos were observed.

A noteworthy point has been the inconstancy of the lesions produced in the membrane. Six eggs inoculated with the same material often produced several excellent membranes but absence of infection was by no means infrequent. Occasionally all inoculated eggs were negative and it was necessary to go back to membrane material stored in Petri dishes in the icebox. This inconstancy persists more than 6 months after the original isolation, and was likewise reported by Torres and Teixeira with alastrim.[4]

The character of the lesions depends on the severity of the infection on the membrane. If 10 to 50 discrete pocks appear, these reach a maximum size of about 1 mm. in about 72 hours; they are raised and rounded, and disappear within 2 or 3 days. When a heavier infection with crowding occurs, the pocks are about 0.5 mm. in diameter. They rapidly coalesce to form a yellowish raised area of necrotic tissue, flat on top and as large as 2 cm. in diameter. This plaque becomes dried and is cast off when the chick hatches. No embryo skin lesions have been noted, although some necrotic areas have been seen in the liver in heavily infected eggs. These pathological changes will be the subject of further study.

The recent work of Craigie and Wishart,[6] on the complement fixation reaction in variola provided a splendid means to confirm the presence of the virus. Membranes of the thirty-sixth passage, cut into small fragments, dried in the Mudd-Flosdorf apparatus, were forwarded to the Connaught Laboratories to be used as antigen. Dr. J. Craigie[7] kindly reported that variola membrane antigen gave complete fixation with an S serum but only partial fixation with an L serum, probably due to deterioration of the L antigen as a result of incomplete drying. The presence of the variola virus was further confirmed by staining impression preparations with Victoria Blue "Bayer", using the method of Herzberg.[8] Paschen granules were readily found in infected membranes, although not in such large numbers as in vaccinal membranes.

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6 Craigie, J., and Wishart, F. O., *Can. Pub. Health J.*, 1936, **27**, 371.

7 Personal communication.

8 Herzberg, K., *Zentralbl. f. Bakt. Abt.* 1, 1936, **136**, 257.

9096 P

Blood Oxygen Changes After Passive Vascular Exercise of the Extremities.

J. Ross Veal and William M. McCord. (Introduced by Howard H. Beard.)

From the Departments of Surgery and Biochemistry, Louisiana State University, School of Medicine.

Alternating negative and positive pressure applied to an extremity produces changes in the blood flow. If the flow is increased beneficial results might be expected. Increase in blood flow should increase the oxygen saturation of the venous blood. Therefore, oxygen saturation determinations from the superficial and deep venous blood of the treated extremity made before and after one hour trial treatment should indicate the degree of increased blood flow. We have applied this test to a series of 18 arteriosclerotic cases presenting vascular symptoms of the lower extremities.

In this series 9 cases presented only exercise pain, 5 cases rest and exercise pain with cyanosis of toes, 3 cases presented dry gangrene of one or more toes, and one case presented only severe night ischemic pain. Each case was studied in the basal state. Samples of blood were drawn from the superficial veins of the dorsum of the foot, or near the ankle, and from the popliteal vein. These samples were collected under oil and the percentage of oxygen saturation was determined by the Van Slyke method. Each patient was then given one hour of alternate suction and pressure therapy, using 4 short cycles per minute. The pressure ranged from minus 80 to plus 20 mm. Hg. Immediately after completion of one hour of treatment samples of blood were collected from the same locations and compared with the first specimens. These patients were then placed on regular treatment of one to 2 hours daily. They received from 16 to 240 hours. The average treatment per patient was 83.6 hours.

We are now able to draw some conclusions as to the clinical results obtained by this form of therapy. We can also correlate these results with the oxygen saturation changes and determine the value of this test as a prognostic aid. Our cases have been placed in 3 groups. In the first group (Table I) are the cases that have been markedly improved or entirely relieved of their symptoms.

As shown in Table I there was a rise in the percentage of oxygen

TABLE I.

Cases Presenting symptoms	% O_2 Saturation Venous Blood: Rest period Superficial	Rest period Deep	1 hr. treatment Superficial	1 hr. treatment Deep	Treatment Hr. per case
Exercise pain	62	50	92	72	190
" "	69	38	75	60	50
Gangrene	43	34	58	49	32
"	55	45	60	60	26
Rest pain	95	28	85	35	126
Exercise pain	59	36	67	—	71

saturation of the superficial venous blood in 5 cases, and a fall in one case. There was an increase in the percentage of oxygen saturation of the deep venous blood in 5 cases, and in one case the percentage was not recorded.

In group II, Table II, are the cases in which clinical improvement followed treatment, but the symptoms have not been relieved.

TABLE II.

Cases Presenting Symptoms	% O_2 Saturation Venous Blood: Rest period Superficial	Rest period Deep	1 hr. treatment Superficial	1 hr. treatment Deep	Treatment Hr. per case
Exercise and rest pain Cyanosis of toes	59	42	69	59	25
Exercise pain	43	43	72	62	145
" "	78	83	83	53	35
Exercise and rest pain Cyanosis of toes	70	30	63	40	16
Exercise and rest pain	79	25	74	25	240
Exercise and rest pain Cyanosis of toes	55	25	55	35	106

As shown by Table II there was a rise in the percentage of oxygen saturation of the superficial venous blood in 3 cases. There was a fall in 2 and one remained the same. There was a rise in the percentage of oxygen saturation of the deep venous blood in 4 cases, a fall in one and one remained the same.

TABLE III.

Cases Presenting Symptoms	% O_2 Saturation Venous Blood: Rest period Superficial	Rest period Deep	1 hr. treatment Superficial	1 hr. treatment Deep	Treatment Hr. per case
Exercise pain	84	57	84	33	145
" "	85	93	85	85	147
" "	72	61	72	61	67
" "	70	42	56	53	42
Exercise and rest pain Cyanosis of toes	53	27	53	27	26
Gangrene of great toe	42	42	51	—	16

In group III, Table III are the cases in which treatment has failed to produce any improvement.

As shown in Table III there was a rise in the percentage of oxygen saturation in only one case. There was a fall in one case and 4 remained the same. There was a rise in the percentage of O_2 saturation of the deep venous blood in one case. There was no change in 2, and a fall in 2. In one case the percentage was not recorded for the deep venous blood after treatment.

These observations show that the oxygen saturation test does *not* offer an absolute standard as to prognosis in treatment of arteriosclerotic vascular disease of the extremities with the alternate suction and pressure method. Generally speaking, however, an increase in oxygen saturation of either the superficial or the deep venous blood, or a rise in both, after one hour trial treatment indicates that some improvement will follow this form of therapy. When there is no change or a fall in the oxygen saturation after one hour trial treatment the prognosis is quite poor. There may be a wide discrepancy between the changes of the oxygen content of the deep and superficial venous blood after treatment with alternate suction and pressure therapy.

9097

Thermotherapy in Experimental Tuberculosis.

EMIL BOGEN.

From Olive View Sanatorium, San Fernando, California.

The possible value of various meteorologic factors in the prevention and treatment of pulmonary tuberculosis cannot be determined with certainty from an analysis of the fluctuating morbidity and mortality rates, which are so much more responsive to other influences.[1] Methods recently used in the attempt to explain the existing variations in the tuberculosis-rates in different parts of the United States on the basis of climatology would give quite different conclusions if applied to the figures of half a century ago.[2]

An enormous clinical experience has yielded a similar diversity of conclusions. Davos and Saranac Lake, Florida and the Riviera, Asheville and Italy, Colorado and Egypt, offer every combination of

1 Evans, G. A., *Handbook of Historical and Geographical Phthisiology*, 1888.

2 Cowles, A., and Chapman, E. N., *J. Am. Stat. Assn.*, 1935, **30**, 517.

altitude, humidity and temperature to the enthusiastic but inconsistent climatologist.[3] Human experiments such as afforded by the group who inhabited Mammoth Cave in Kentucky[4] or a similar group in Alaska[5] have been likewise inconclusive. The effects of artificial hyperpyrexia[6] in the treatment of pulmonary tuberculosis still lack confirmation. Animal experiments[7, 8] have repeatedly disproved the reported[9] beneficial results of briefly exposing tuberculous animals to high temperatures. Induction of fever by the administration of dinitrophenol, 5 mg. 3 times a week,[10] or by exposure to the general or local condenser-field or the electromagnetic field of 6-meter wavelength 1500-watt generator twice weekly for 10-minute periods[11] were similarly without demonstrable effect on the course of experimental tuberculosis in guinea pigs.

On the other hand, the exposure of infected guinea pigs to continuous warmth throughout the period of infection has given definite and surprisingly good results. So far, 225 guinea pigs, kept in 45 cages, at a temperature of over 80°F., and usually between 85 and 90°, at the Olive View Sanatorium for 3 months after inoculation with from 0.1 to 0.0001 mg. of the virulent human type tubercle bacilli H37, H98, or H115 during the past 5 years have shown, on the average, just half as much tuberculosis when necropsied at the end of 3 months as did 150 guinea pigs similarly inoculated at the same time and kept in 30 cages at outside temperature. Only once did a set of guinea pigs kept in a warm cage show more tuberculosis than did the controls, while 44 different sets of 5 guinea pigs kept in the warm room showed less.

The average amount of tuberculosis, on the scale of 16 units per pig, or 80 per cage, as described previously,[12] was 32 units for the controls and 16 units per cage for the animals kept in the warm cages, a reduction of 50% in the amount of tuberculosis found at autopsy in the treated cages as compared with the controls. The average difference of 16 units per cage, with a probable error of less than 2.5, means that the difference here found is more than 6 times the

3 Flick, L., *Trans. Nat. Tuberc. Assn.*, 1906, **2**, 442.

4 Croghan, *Boston M. and S. J.*, 1843, **28**, 188.

5 Welzl, Jan, *Thirty Years in the Golden North.*

6 Duncan, G. R., and Mariette, E. S., *Am. Rev. Tuberc.*, 1935, **31**, 687.

7 Corper, H. J., and Gauss, J., *Am. Rev. Tuberc.*, 1920, **4**, 269.

8 Rogers, J. B., *Am. Rev. Tuberc.*, 1922, **6**, 119.

9 Murphy, J. B., and Sturm, E., *J. Exp. Med.*, 1919, **29**, 1, 35.

10 Cutting, W. C., Mehrtens, H. G., and Tainter, M. L., *J. A. M. A.*, 1933, **101**, 193.

11 Kling, D. H., and Rubin, H. M., *Am. Rev. Tuberc.*, 1936, **34**, 498.

12 Bogen, E., *Trans. Nat. Tuberc. Assn.*, 1932, **28**, 163.

probable error of such figures, beyond the range of reasonable probability that this might have been a mere chance variation.

Guinea pigs in 32 of the 45 cages kept in the warmth had been inoculated subcutaneously, while in 13 they had inhaled a spray of the bacilli. The average difference between the warm cages and the controls among those subcutaneously inoculated was 18, while among those infected by inhalation the average difference was 10; but in both cases the differences appear to be significant, the lower figure among the inhalation-cages probably reflecting the greater natural variation encountered with this type of infection.

The temperature in these cages has been automatically maintained within a few degrees of the desired point by a thermostatic control operating electric lamps or resistance-coils. Temperatures above 95°F. proved fatal to the animals, while the animals kept constantly at 48°F. and at 70°F. failed to show the retardation in the development of tuberculosis noted in those kept at a warmer temperature. No definite differences could be seen, however, between the results obtained at 80-85°, 85-90°, and 90-95°F. During the summer it was necessary to cool the cages artificially during the daytime, which was done by opening the doors and by the use of ice.

In order to exclude the action of luminous or ultra-violet or infra-red light as a factor in these results, cages were heated by convection from a concealed electric heater, and shut off from outside light, but the beneficial effect of heat was observed just as in those exposed to the light of an incandescent electric bulb or to diffused sunlight. It may be noted, however, that previous studies in this laboratory[12] have failed to show significant effects of darkness, ultraviolet, overcrowding, dampness or other environmental factors on the development of experimental tuberculosis in guinea pigs.

Twelve of the cages, comprising 60 guinea pigs, were subjected to the warm treatment for only half of the 3 months' duration of the infection. Half of these were removed from the warm cages to the outside air after 6 weeks of warmth, while the other half were first placed in the warm cages 6 weeks after they had been infected. In both groups, the development of tuberculosis was definitely inhibited, showing no significant difference between those receiving the warmth early or later in the course of their infection. But the amount of tuberculosis developing, though less than that in the controls, was in each case greater than that developing in cages of guinea pigs which were exposed to the warmth for the entire 3 months.

TABLE I.
Thermotherapy in Experimental Tuberculosis.

Date of Infection	Route	Months treated	Temp. °F.	No. Cages treated	Aver. Amt. Tuberculosis in treated	No. Cages Controls	Aver. Amt. Tuberculosis in Controls	% Difference from Controls
10/1/31	S.C.	3	85	1	16	1	43	—63
3/4/32	,,	,,	85	1	28	1	47	—40
6/7/32	,,	,,	90	1	27	3	38	—29
10/9/32	,,	,,	90	1	25	2	39	—36
1/25/33	,,	,,	90	1	34	2	25	+36
1/26/34	,,	,,	90	1	16	2	29	—45
5/9/34	,,	,,	90	1	24	2	28	—14
1/21/35	,,	,,	85	4	12	2	33	—66
,,	,,	,, B	85	1	33	A	33	0
6/10/35	,,	,,	85	5	19	2	36	—46
,,	,,	,, C	85	1	15	A	36	—58
1/15/36	,,	,,	85	3	17	4	35	—52
,,	,,	,,	80	1	13	A	35	—64
,,	,,	,,	90	1	7	,,	35	—80
,,	,,	,, D	85	1	15	,,	35	—57
7/17/36	,,	,,	85	6	12	3	27	—55
,,	,,	1½	85	3	9	A	27	—67
1/15/36	Inhaled	3	85	4	14	3	27	—48
7/17/36	,,	,,	85	3	14	3	22	—36
,,	,,	1½	85	6	17	A	22	—23
10/1/31	S.C.	3	48	1	35	A	43	—18
6/7/32	,,	,,	48	1	43	,,	38	+13
1/25/33	,,	,,	48	1	18	,,	25	—28
6/10/35	,,	,,	48	1	27	,,	36	—25
1/15/36	,,	,,	48	1	27	,,	35	—23
,,	,,	,,	70	1	41	,,	35	+17

A = recorded on preceding line. B = Beryllium. C = Cod Liver Oil. D = ½ diet.

Inasmuch as it was suggested that the effect of the warmth might be to conserve caloric expenditure, and thus improve the animals' nutrition, one cage of animals was maintained in the warm temperature on only half of the usual ration of food. This had absolutely no effect on the beneficial effect of the warm temperature, the cage of animals receiving the half-diet showing just about the same amount of tuberculosis as those on full diet, whether in the warm cages or in the controls (D). Another cage of animals receiving cod-liver oil in addition to the warm temperature also showed no difference from the animals warmed on the regular diet (C), again confirming the failure to find effect of cod-liver oil on experimental tuberculosis in guinea pigs previously experienced.

On the other hand, the repeated injections of beryllium, as previously reported,[13] not only increased the amount of tuberculosis developing in the infected animals at room temperature, but when administered to animals in the warm cages caused them to develop just about the same amount of tuberculosis as the untreated controls (B), in other words, the deleterious effect of the beryllium exactly balanced the beneficial effect of the warmth.

Three different strains of guinea pigs were used for these experiments, the cross bred Olive View stock and the 2 highly inbred families, family 13 and family 39, which were studied many years ago by Lewis and Wright. All 3 families seemed to respond similarly to the warm temperatures, with the development of lower amounts of tuberculosis than when at room temperature.

The mechanism by which this retardation of the development of tuberculosis in infected guinea pigs is brought about is still obscure. No demonstrable increase in body-temperature could be noted in the animals kept in the warm cages, as compared with the controls, both averaging 101°F. rectally. Blood studies for possible leukocytosis or lymphocytosis as a result of the warmth have been, so far, inconclusive. Although, as might be expected, the infected animals, kept from developing so much tuberculosis by the warmth, did show considerably better weight curves than those in which the disease advanced more extensively, healthy animals placed in the warm cages showed no greater gain than did healthy animals kept under control conditions.

One possible interpretation of the results is suggested by the behavior of the animals under these conditions. The guinea pigs in the warm cages appear to be uncomfortable if they move, and so tend to remain much more quiet than do the controls. In other words, they are here subjected to an enforced intensive rest treatment, from

[13] Loomis, R. N., and Bogen, E., *Am. Rev. Tuberc.*, 1935, **32**, 475.

which they stir only for eating and other functions, or when alarmed. The animals, remaining more quiet because of the discomfort induced by movement in the warm cages, may thereby be spared some of the spread of the disease secondary to muscular activity, and the increased lymphatic and circulatory drainage that such activity induces, and thus show a more marked localization of the disease. It has been impossible for us so far to determine what effect rest obtained in any other way might have on the course of experimental tuberculosis in guinea pigs, but we cannot deny the possibility of such benefit.

This simple explanation, if true, would obviate any practical application of the warmth treatment, or moderate thermotherapy, in the treatment of pulmonary tuberculosis in man, in whom rest may be achieved by other, perhaps more pleasant means, but furnishes additional evidence for the generally accepted belief that rest is an important factor in aiding recovery from tuberculous infection.

9098 P

A Cinematic Study of Bronchiolar Reactions.*

TORALD SOLLMANN AND A. J. GILBERT.

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A cinematic record was shown of directly observed bronchiolar reactions. These were obtained by applying drugs to microscopic cross-sections of fresh bronchioles, and photographing through a low-power microscope.

Method. The excised lungs are filled with a warm solution of 10% gelatin in Ringer's, by intratracheal injection. The gelatin is hardened by placing the lungs in iced Ringer's, where excitability of the muscle is retained for several days. Free-hand sections are made with a razor. Each section is pinned onto a perforated piece of cork attached to the bottom of a Petri dish, and the dish is filled with Ringer's solution. The dish is placed on the warm-stage of a microscope, and drugs applied after a preliminary warming, approximately to body temperature, for at least 30 minutes. Reactions are recorded either by means of camera lucida drawings, or, as in this case, by taking cinematic records.

* This work was aided by a grant from the Therapeutic Research Committee of the Council on Pharmacy and Chemistry of the American Medical Association.

Results. 1. *Cilia.* With this method, the cilia are very active in sections less than 8 hours old. After this time, the activity decreases or stops. With active cilia, most autonomic drugs have no visible effect. Nicotine 1:2500 has no effect; but 1:500 causes stoppage of cilia. Saturation of the Ringer's solution with chloroform causes marked decrease or stoppage of ciliary activity after about 20 minutes. Cyanide causes stoppage of cilia in one to 3 minutes.

2. *Rhythmic bronchiolar contractions.* These occur spontaneously in a large proportion of dog (puppy) bronchioles, and have also been observed in the cat, rabbit and rat. They are best brought out by keeping the preparation warm for an hour or two, and are usually augmented by a small amount of mecholyl (ca. 1:1,000,000). They may be pendular or tonic, or pendular superimposed on tonic. The rate of the pendular contractions sometimes approximates the normal respiratory rate.

Using short longitudinal sections of bronchioles, definite rhythmic peristaltic waves have been produced by mecholyl (sometimes followed by epinephrine) in the dog and cat. The waves travel sometimes peripherally and sometimes centrally, even in a single section.

3. *Drug Reactions.* Work with drugs has been for the most part qualitative, since the reactions of thin sections would hardly be comparable quantitatively with reactions of intact bronchioles. *Constrictors* include mecholyl (acetyl-β-methyl-choline chloride), histamine, barium, physostigmine, pilocarpine, cyanide, and nicotine, in about the order of their effectiveness. Histamine and mecholyl produce marked contraction of dog bronchioles in concentrations of 1:1,000,000. Of the *dilators,* atropine is highly effective against mecholyl, physostigmine, and pilocarpine; and less effective against histamine contraction. Epinephrine is generally effective against histamine and mecholyl (using cat and dog bronchioles). Magnesium is effective against barium. Papaverine is effective against most constrictors. Chloroform and ether are relatively ineffective.

4. *Anaphylaxis.* Egg albumin has no effect on normal bronchioles, but produces a marked contraction of bronchioles of rabbits sensitized 3 weeks previously by one cc. of 10% egg albumin hypodermically. The action of drugs on sensitized bronchioles does not differ materially from that on normal bronchioles.

5. *Limitations.* The guinea pig, which has been a favorite subject for the study of anaphylaxis and bronchiolar reactions, is

thus far unavailable for this method, since the sections contract shut as soon as the gelatin is melted from the lumen, and are not relaxed by any of the known dilators. Tentatively, this effect is attributed to the action of elastic tissue in the bronchial wall.

Conclusion. Fresh sections of excised lung may be used for microscopic study of ciliary activity, rhythmic contractions, peristalsis, and drug and anaphylactic reactions of bronchioles.

9099

A Method for Studying Changes in Diastolic Resistance to Blood Flow in the Coronary Arteries.

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In a series of recent articles[1, 2, 3] the blood flow from moment to moment in the various branches of the coronary arteries has been established by constructing differential pressure curves from a combination of the central and peripheral coronary pressure curves (after the latter had been raised to their proper ordinate value). The method, however, is time consuming and what is more important, may miss altogether very rapid and transient changes in coronary flow such as, for example, might follow nerve stimulation, since the necessary data for constructing a flow curve can not be obtained in less than 10-20 heart beats.

To offset these drawbacks, a simple method (requiring the registration of only 1-2 heart beats) is suggested for determining changes in diastolic resistance and in most cases this can be used as an index of qualitative changes in coronary diastolic blood flow.

The experimental setup and method are as follows: After opening the chest and pericardium of an anesthetized dog, optical records of aortic and coronary pressures are taken by inserting manometers of adequate frequency into the aorta through the subclavian artery and into a suitable side branch of a coronary artery. An electromagnetic clamp is placed on the coronary artery central to its recording

[1] Gregg, D. E., Green, H. D., and Wiggers, C. J., *Am. J. Physiol.*, 1935, **112**, 362.

[2] Green, H. D., Gregg, D. E., and Wiggers, C. J., *Am. J. Physiol.*, 1935, **112**, 627.

[3] Gregg, D. E., *Am. J. Physiol.* In press.

manometer. The heart is driven by an artificial pacemaker connected to the right auricle. The clamp and pacemaker are so synchronized (by a device to be described later) that the artery is clamped in successive beats only during the latter part of diastole. Since it has already been established (1) that the diastolic coronary resistance remains unchanged during the latter portion of diastole, then the rate of pressure drop in the artery (obtained by drawing a tangent to the recorded pressure curve at an arbitrary pressure point) can be utilized as an index of the prevailing diastolic resistance. This measure of coronary diastolic resistance taken together with the central coronary or aortic pressure will in most cases give adequate information for comparing qualitatively diastolic flows under different experimental conditions.

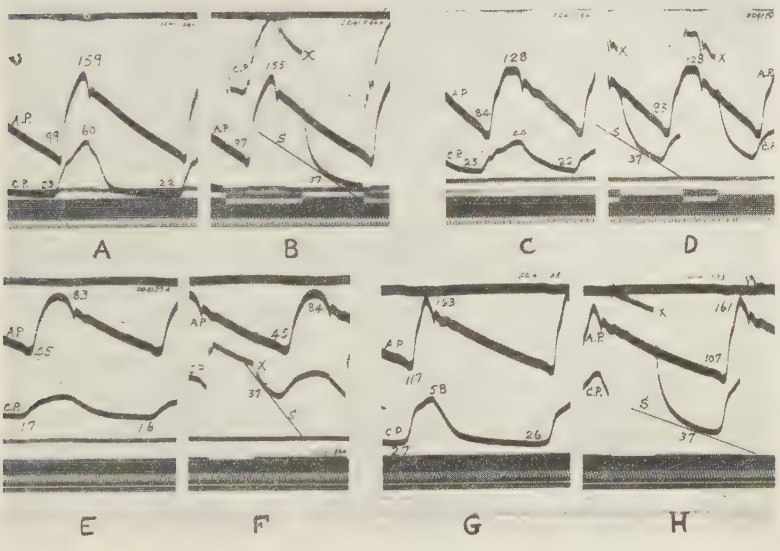

FIG. 1.

Curves illustrating rates of diastolic runoff B, D, F, H and actual coronary diastolic resistance, A, C, E, G, in ramus descendens anterior. A.P., aortic pressure; C.P., coronary pressure; X, time of clamping; S, slope line. Time, 0.02 second.

Figure 1 presents typical records, all taken from the same experiment. In records B, D, F, H, the ramus descendens anterior was mechanically clamped at the time marked X and the diastolic slopes determined at 37 mm. Hg. (this pressure being arbitrarily chosen). The slope lines, S, were then drawn. To check the use of such slopes as a criterion of diastolic resistance, the peripheral coronary

resistances have been recorded in records A, C, E, G (after permanently clamping the coronary artery central to the recording manometer until diastolic equilibrium was attained). These curves were obtained within a few heart beats of records B, D, F, H.

Records B, D may first be compared. In such records with aortic blood pressures of 155/97 and 128/83 mm. Hg. respectively, the diastolic slopes at 37 mm. Hg. are identical. This necessitates that the peripheral coronary diastolic resistances also agree. Inspection of records A, C shows that each diastolic peripheral pressure has a value of 22 mm. Hg. giving a resultant effective pressure of 15 mm. Hg. in each case. It is obvious that this agreement of diastolic slopes does not necessarily mean that the diastolic blood flows are the same, for the peripheral coronary pressure is only one of the determinants of blood flow. When the aortic head of diastolic pressure is also considered, the diastolic coronary flow is greater in B than in D (effective pressure at the end of diastole of 75 mm. Hg. as compared with 61 mm. Hg.).

In F as compared with H is shown the effect on coronary diastolic resistance and also flow of raising the aortic pressure by moderate compression of the aorta. In F, the aortic pressure is 84/45 mm. Hg. and the diastolic slope at 37 mm. Hg. is greatly in excess of that in B and D. This is in keeping with the much lower diastolic resistance of 16 mm. Hg. in E. In G the aortic pressure has been raised by aortic compression to 163/117 mm. Hg. This results in a higher diastolic resistance of 26 mm. Hg. and markedly lowers the rate of diastolic runoff as evidenced by the decreased slope S, in H. The diastolic blood flow is here much greater than in F (also D) because the difference between the aortic and peripheral diastolic pressure is much greater. The relative diastolic blood flow in B as compared with H cannot be predicted from the data at hand.

Such records, then, indicate that the gradient of pressure decline in a peripheral coronary artery clamped during the latter part of diastole can be used as a simple and accurate index of coronary diastolic resistance to blood flow under variable physiological conditions such as blood pressure, pulse pressure, heart rate, and nerve stimulation. In conjunction with the central coronary pressure, it can at times serve as a qualitative index of diastolic coronary blood flow.

9100 P

Effect of CO and Methylene Blue on Respiration of Embryos.*

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Barron *et al.*[1] have adduced evidence in support of the hypothesis that methylene blue and other dyes stimulate the oxygen consumption of certain cells by promoting the oxidation of carbohydrates or some of their degradation products. They suggest, moreover, that the catalytic process depends upon the reversible reduction and reoxidation of the dyestuff and that this is independent of the function of the respiration enzymes, for when these are poisoned by cyanide and carbon monoxide the addition of methylene blue or other dyes restores respiration to the normal levels.

Recently Reid[2] has shown that leuco-methylene blue is not autoxidizable, as is commonly supposed, but that the process involves a metal catalysis and is sensitive to CO poisoning. Cook, Haldane and Mapson[3] have demonstrated a CO sensitivity of methylene blue stimulated oxidations in *B. coli.* Likewise, Chang and Gerard[4] have shown that the stimulation of nerve respiration by cresyl blue is depressed by CO. We[5] have previously pointed out that the methylene blue stimulation of respiration in diapause (blocked) grasshopper embryos (*Melanoplus differentialis*) is sensitive to CO, whereas the normal respiration of such embryos is little if at all affected by this substance. In actively developing embryos the stimulation due to methylene blue as well as a large fraction of the normal respiration is depressed by CO. Our inability to obtain antagonism by methylene blue of cyanide inhibition of respiration except in cases where very low concentrations of cyanide were used and the results obtained by other workers as well as ourselves on the

* Aided by a grant from the Rockefeller Foundation for research in cellular physiology.

1 Barron, E. S. G., and Harrop, G. A., *J. Biol. Chem.*, 1928, **79**, 65; Barron, E. S. G., *J. Biol. Chem.*, 1928, **81**, 445; Barron, E. S. G., and Hoffman, L. A., *J. Gen. Physiol.*, 1930, **13**, 483; Barron, E. S. G., and Hamburger, M., Jr., *J. Biol. Chem.*, 1932, **96**, 299; DeMeio, R. H., Kissin, M., and Barron, E. S. G., *J. Biol. Chem.*, 1934, **107**, 579.

2 Reid, A., *Berichte*, 1930, **63**, 1920; *Bioch. Z.*, 1930, **228**, 487.

3 Cook, R. P., Haldane, J. B. S., and Mapson, L. W., *Bioch. J.*, 1931, **25**, 534; *Bioch. J.*, 1931, **25**, 880.

4 Chang, T. H., and Gerard, R. W., *Am. J. Physiol.*, 1931, **97**, 511.

5 Bodine, J. H., and Boell, E. J., Proc. Soc. Exp. Biol. and Med., 1936, **34**, 629; *Physiol. Zool.*, 1936 (in press).

CO sensitivity of methylene blue stimulation strongly suggests that in some living systems methylene blue catalysis of respiration depends upon and functions through the normal respiratory mechanism of the cells.

The present communication is designed to furnish evidence that this suggestion is to some extent tenable. Techniques involved in respiration studies on embryos as well as the characteristics of the embryos themselves have been described previously.[5, 6]

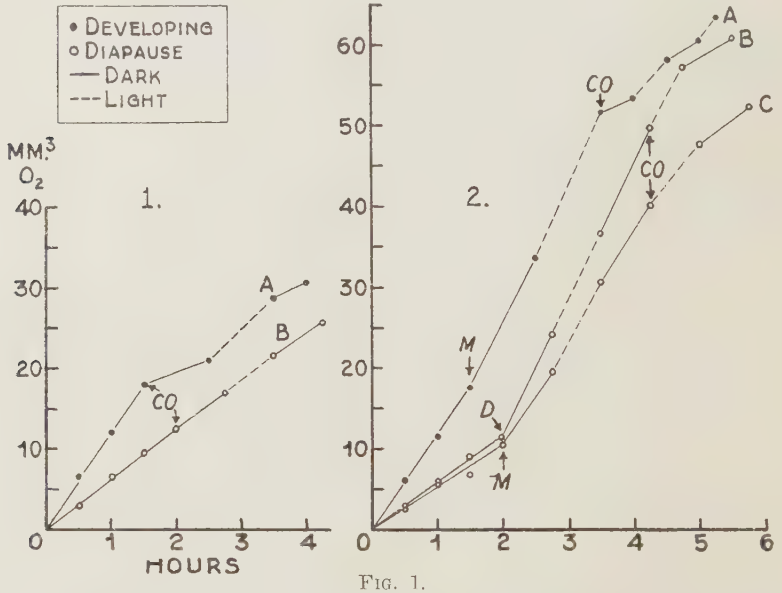

FIG. 1.

Comparison of the effect of CO on respiration of developing (A) and diapause (B) grasshopper embryos in dark and light. $CO/O_2 = 95/5$ added at arrows. Ordinate—O_2 uptake per 100 embryos; abscissa—time in hours.

FIG. 2.

Showing methylene blue stimulation of respiration and the reversible effect of CO in dark and light on developing (A) and diapause (C) embryos. Curve B shows similar effects of CO on stimulation of respiration by DNP. Concentration of methylene blue 12.6×10^{-5} molar, $CO/O_2 = 95/5$. Methylene blue added at arrow with M; DNP, at arrow with D. Ordinate and abscissa same as in Figure 1.

The essential features of the present work are depicted in the typical graphs shown in Figs. 1 and 2. The differential susceptibility of diapause and developing embryos to CO is shown graphically in Fig. 1. In developing embryos the CO inhibition is considerably diminished by light (450 W. tungsten filament lamp through glass-sided bath). Respiration in CO in the dark is 25% and in the light 75% of the normal. Curve A, Fig. 2, shows that the

[6] Bodine, J. H., and Boell, E. J., *J. Cell. and Comp. Physiol.*, 1936, **8**, 357.

oxygen uptake of developing embryos in the presence of methylene blue is slightly greater in the light than in the dark. This increase is observed also in the case of diapause embryos (curve C) and may be attributed to the photodynamic action of methylene blue.[7] With dinitrophenol no such effect is noted. It is apparent from the curves of Fig. 2 that CO inhibits the methylene blue-stimulated respiration in diapause and developing embryos as well as the normal oxygen uptake of developing embryos (Fig. 1). Moreover, the inhibition is made reversible by light.

It has been pointed out by DeMeio and Barron[8] and confirmed by Krahl and Clowes,[9] and by Bodine and Boell[10] that DNP stimulates respiration by functioning through the normal oxidase-dehydrase systems of the cell. Thus any interference with the activity of the oxidase involved (as for example by KCN or CO) would restrict to a considerable extent the increased oxygen uptake induced by DNP. In grasshopper embryos methylene blue stimulation and DNP stimulation both appear to be similarly limited by CO. This fact, then, suggests that they both function through, although not necessarily in the same way, a CO sensitive mechanism whose affinity for CO is greatly reduced in the light.

9101

Analysis of the Acid-soluble Phosphates of Muscle Following the Injection of Glucose Plus Insulin.

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The following observations have been made in regard to changes in inorganic phosphate of plasma.[1] 1. Injection of insulin, while causing a lowering of plasma phosphate in normal rabbits, had little or no effect on plasma phosphate in adrenalectomized rabbits. 2. Injection of epinephrine was equally effective in normal and adrena-

7 Blum, H. F., *Cold Spring Harbor Symp. Quant. Biol.*, 1935, **3**, 318.

8 DeMeio, R. H., and Barron, E. S. G., PROC. SOC. EXP. BIOL. AND MED., 1934, **32**, 36.

9 Krahl, M. E., and Clowes, G. H. A., *J. Biol. Chem.*, 1935, **111**, 355.

10 Bodine, J. H., and Boell, E. J., PROC. SOC. EXP. BIOL. AND MED., 1936 (in press).

1 Cori, C. F., and Cori, G. T., *Arch. Exp. Path. Pharm.*, 1933, **172**, 249.

lectomized rabbits in causing a decrease in plasma phosphate. 3. Injection of glucose plus insulin—in contrast to insulin alone—resulted in a definite fall in plasma phosphate in adrenalectomized rabbits.

As pointed out previously,[1] two mechanisms appear to be in operation. One is associated with hypoglycemia and reflex discharge of epinephrine and is seen in fasting animals after insulin injections, epinephrine causing an accumulation of hexosemonophosphate in muscle at the expense of inorganic phosphate.[2, 3] After adrenalectomy this mechanism is no longer in operation and insulin alone, in the absence of an abundant supply of glucose, causes little or no change in blood inorganic phosphate, an observation which has recently been confirmed by Pijoan and Quigley[4] on adrenalectomized dogs. The second mechanism, the nature of which has not been explained, may possibly be associated with glycogen storage in the tissues.[5] It is seen in both normal and adrenalectomized animals after glucose administration, to which an insulin injection may be added, should the endogenous supply not be sufficient to cause a rapid disappearance of the injected glucose.[1] That the latter condition is essential is shown by the observation that glucose has hardly any effect on blood inorganic phosphate in depancreatized dogs.[6]

It seemed possible that during the rapid disappearance of glucose one of the known acid-soluble phosphate compounds may temporarily accumulate in the tissues and thereby cause a decrease in inorganic phosphate. The hexosemonophosphate content of muscle did not change after glucose administration,[2] but the other phosphate compounds, known to occur in muscle, had not yet been analyzed under these conditions. This was done in the present experiments.

In Table I are recorded experiments on cats. Enough glucose was administered in each case to prevent the development of hypoglycemia due to the injection of insulin. At a time at which the inorganic phosphate of plasma had fallen considerably below the initial value, the hexosemonophosphate content of muscle remained substantially unchanged (confirming similar experiments on unanesthetized rats[2] in which, however, blood inorganic phosphate had not been determined). Hydrolysis curves in N HCl (see second part of Table I), before and after injection of glucose plus insulin,

2 Cori, C. F., and Cori, G. T., *J. Biol. Chem.*, 1931-32, **94**, 581.

3 Cori, G. T., and Cori, C. F., *J. Biol. Chem.*, 1936, **116**, 119.

4 Pijoan, M., and Quigley, T. B., PROC. SOC. EXP. BIOL. AND MED., 1936-37, **35**, 131.

5 Cori, C. F., *Physiol., Rev.*, 1931, **9**, 143.

6 Bolliger, H., and Hartman, F. W., *J. Biol. Chem.*, 1925, **64**, 91.

TABLE I.

Effect of Injection of Glucose Plus Insulin on Acid-Soluble Phosphate of Muscle in Amytalized Cats. All values are given in mg. %.

	Before injection						1 to 2 hrs. after injection (1 to 1.3 gm. glucose per kilo per hr., 10 units insulin)					
	Plasma		Muscle				Plasma		Muscle			
Exp. No.	Inorg. P	Sugar	Lactic acid	Hexosemonophosphate: Hexose	Hexosemonophosphate: P found	P calc.	Inorg. P	Sugar	Lactic acid	Hexosemonophosphate: Hexose	Hexosemonophosphate: P found	P calc.
1	4.8	134		25			3.9	243		23		
2	3.8	148	21	27	4	5	2.0	108	52	25	4	4
3	5.2	139	20	32	5	6	3.4	280	38	33	5	6
4	6.9	147	15	33	5	6	2.8	204	31	36	6	6
5	4.7	103					2.3	366				
6	5.4	165					2.5	201				

	Before injection							1 to 2 hrs. after injection (1 to 1.3 gm. glucose per kilo per hr., 10 units insulin)						
	Muscle							Muscle						
	Inorg. P	Phosphocreatine P	Hydrolysis in N-HCl: 0	10	30	180 min.	Total P	Inorg. P	Phosphocreatine P	Hydrolysis in N-HCl: 0	10	30	180 min.	Total P
3			107	152	154	163	169			106	148	150	165	170
4			104	142	146	166*	169			108	147	153	171*	173
5		76†	105						74†	109				
6	18‡	82	100				177	22‡	83	105				179

* Hydrolyzed for 18 hr.

†Method of Fiske and Subbarrow

‡Method of Sacks and Sacks.

TABLE II.

Effect of Injection of Glucose Plus Insulin on Acid-Soluble Phosphate of Muscle in Amytalized Rats. All values are given in mg. %.

Before injection					40 minutes after glucose plus insulin					
		P after hydrolysis in N-HCl					Hydrolysis in N-HCl			
Inorg. P	Phospho-creatine P	0	10 min.	Total P	Inorg. P	Phospho-creatine P	0	10 min.	Total P	Plasma P
25	69	94	134	168	25	67	92	136	173	5.4*
26	64	90	131	165	23	59	82	126	167	3.9
25	69	94	133	166	26	66	92	132	169	3.9
28	67	95	135	174	24	68	92	133	175	4.2

*No injection.

did not give an indication of a change in the distribution of the various acid-soluble phosphate compounds of muscle. Since the zero value of the hydrolysis curves represents the sum of inorganic and phosphocreatine P, there remained the possibility of an increase in phosphocreatine at the expense of inorganic phosphate. In 2 experiments on cats (Table I) and 3 experiments on rats (Table II) an increase in phosphocreatine P could not be detected though there was, at least in 2 experiments, a decrease in inorganic phosphate.

Summary. The decrease in the inorganic phosphate content of plasma following the injection of glucose plus insulin is not accompanied by a detectable increase in the hexosemonophosphate, adenosinetriphosphate or creatine phosphate content of muscle.

9102

Changes in Renal Blood Flow in Relation to Changes in Pressure in Urine Formation.

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In conjunction with certain experiments on the mechanism of chloride reabsorption by the tubules of the kidney of the dog in a heart-lung perfusion system as described by Verney and Starling,[1] we have had occasion to observe some interesting relations between renal blood flow, perfusion pressure, chloride concentration and total chloride excreted in the urine.

Winton[2] has pointed out that the rate of blood flow through the kidney is a factor of secondary importance, as compared with the glomerular filtration pressure, in determining the character and quantity of urinary secretion. Numerous observations have indicated to us an indubitable relation, nevertheless, between renal blood flow and urinary secretion, particularly in the case of spontaneous changes in the former.

Figure 1 shows graphically the results of a type of experiment in which concomitant with a rising renal blood flow, from 110 to 210 cc. per minute, while the perfusion pressure was kept substantially constant for 35 minutes, there was an increase in urine flow from

1 Verney, E. B., and Starling, E. H., *J. Physiol.*, 1922, **56**, 353.

2 Winton, F. R., *J. Physiol.*, 1931, **73**, 151.

0.35 cc. to 4.8 cc. per minute, and an increase in chlorides from 50 to 150 mg. %. This sort of behavior has been noted in a number of instances after the kidney has begun to secrete well.

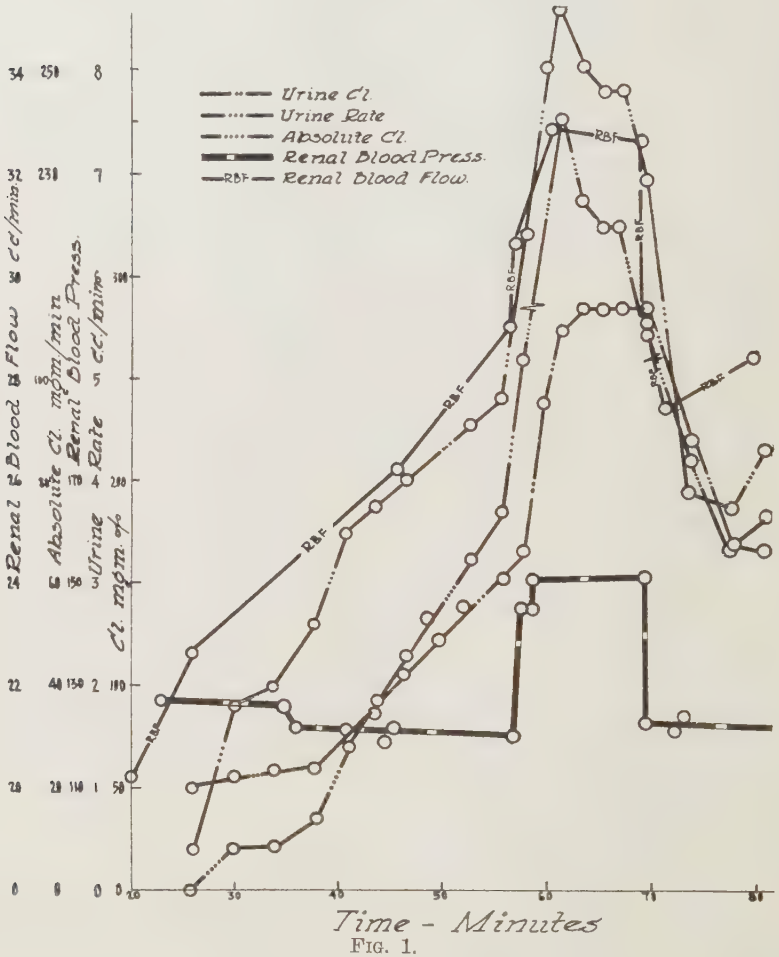

FIG. 1.

These observations are not in any sense contradictory to those of Winton, because he was dealing with changes induced in the rate of blood flow by altering the pressures in the renal vein and the ureter. Moreover, we believe that our observations can be interpreted best by ascribing the results to changes in the state of constriction of certain portions of the vascular bed in the kidneys resulting in changes in glomerular filtration pressure as outlined below.

An increase in the rate of renal blood flow at constant perfusion pressure could result only from a vasodilation in some regions of the

vascular bed of the kidney. This could conceivably be in the afferent arterioles, the efferent arterioles or in the veins.

The evidence from the changes in urinary secretion gives a clue as to the site of the vascular dilatation. With the increase in renal blood flow there were observed large increases in the volume of urine formed and in its chloride content. These increases bespeak an increased glomerular capillary pressure, bringing about augmented filtration. Tubular activity is largely independent of blood flow and pressure (Winton[2]), therefore with increased glomerular filtration the chloride content of urine is increased. With constant renal arterial pressure an increase in glomerular capillary pressure along with an increased blood flow could not result from dilatation of any blood vessels in the kidney except the afferent arterioles. Dilatation of either the efferent arterioles or the veins would result in a decreased glomerular capillary pressure. Thus the changes observed seem to be due primarily to a dilatation of afferent glomerular arterioles.

In the portion of the experiment shown in Fig. 1 from the period 55 to 70 minutes, the perfusion pressure was increased from 80 to 150 mm. Hg. This resulted in an increase in urine flow from 4.8 to 8.5 cc. per minute, and in chloride concentration, from 150 to 280 mg. %. One sees illustrated here the fact described by Canny, Verney, and Winton[3] that with increased rates of glomerular filtration the tubular reabsorption of chloride does not keep pace, and consequently the chloride concentration in the urine rises. The reabsorption of chloride falls behind the net rate of reabsorption of water, otherwise only the absolute chloride elimination and not the chloride concentration would rise.

The absolute chloride excretion rose 300%, considerably more than the chloride percentage, but the latter increased nearly 100%. The tubules obviously do not have a capacity to maintain constant concentration ratios for chloride at varying rates of formation of glomerular filtrate.

These observations on the effect of increased arterial pressure on the composition and volume of the urine are in support of some of the preceding arguments, in particular regarding the interpretation of simultaneous increases of volume and chloride content.

Twenty-five successful heart-lung kidney experiments have been conducted and the type of spontaneous change described has been encountered in one-fourth of the cases. The interpretation of these spontaneous changes is particularly important, we believe, because

3 Canny, A. J., Verney, E. B., and Winton, F. R., *J. Physiol.*, 1930, **68**, 333.

the extreme hypotonicity of heart-lung-kidney urine, and its low chloride content, have been difficult to interpret. The urine in this preparation differs in chloride content so markedly from that in the intact animal that the reasons for the difference must be elucidated before the isolated perfused kidney results can be applied to normal renal physiology. The findings reported here indicate that the hypotonic urine may be due, in part, to a relatively low glomerular filtration rate caused by greater constriction in afferent than in efferent arterioles.

9103

A Comparison of Some Methods for the Extraction of Vitamin B_1 from International Standard Acid Clay.

W. L. SAMPSON AND J. C. KERESZTESY. (Introduced by A. N. Richards.)

From Merck Institute of Therapeutic Research, Rahway, New Jersey.

Williams and co-workers[1] in their description of an improved method for obtaining crystalline vitamin B_1 from rice polish have stated that by using barium hydroxide to extract the vitamin from activated fullers' earth no more than a 50% recovery could be obtained. For this reason they chose to use quinine sulfate to displace the vitamin from fullers' earth. Recently Kinnersley and Peters[2] have reported that by the use of a baryta extraction method, vitamin B_1 can be quantitatively removed from the acid clay used as the international standard. However, they made no comparison of the baryta method of extraction with the quinine extraction method proposed by Williams. In this communication we present the results of our comparison of the Williams' extraction method with other proposed methods together with some data on the potency of crystalline vitamin B_1 in terms of international units.

The method of assay used throughout this work was the Ammerman and Waterman[3] modification of the Smith curative procedure. In most cases the test substance was injected subcutaneously since as reported by Kinnersley and Peters and confirmed by us there is no appreciable difference in the response of the animal to oral or

1 Williams, Waterman and Keresztesy, *J. Am. Chem. Soc.*, 1934, **56**, 1187.

2 Kinnersley and Peters, *Biochem. J.*, 1936, **30**, 985.

3 Ammerman and Waterman, *J. Nutrition*, 1935, **10**, 25.

subcutaneous administration. We considered as the curative dose that amount of substance which would produce complete relief of severe symptoms in 60-80% of the test animals and keep these animals free from convulsions for 4 or more days.

Extractions of the acid clay were made using the Williams method, the Kinnersley and Peters method and an alkaline methyl alcohol method suggested by Smith and Seidell.[4] These extracts together with the untreated acid clay and crystalline vitamin B_1 were administered to polyneuritic rats. The results are given in Table I.

TABLE I.
Curative Dose of International Standard, Extracted and Unextracted, and of Crystalline Vitamin B_1.

Substance	Form administered	Curative dose	No. rats used	No. rats cured	% cured
		mg.			
Int. St'd for B_1	Untreated	20	25	17	68
" " " "	Extracted—M. I. Smith technic	20	9	6	67
" " " "	" Kinnersley & Peters	18	10	6	60
" " " "	" Williams *et al.*	10	30	19	63
Crystalline B_1	Aqueous solution	0.005	55	40	73
" "	Quinine sulfate solution	0.005	20	15	75

It is apparent that according to our criterion the curative dose of the international standard is 20 mg. Likewise the curative doses of the extracts prepared either by the baryta method of Kinnersley and Peters or by the alkaline methyl alcohol method of Smith are approximately the equivalent of 20 mg. of the acid clay. These results are in close agreement with the published findings of these investigators. However, when an extract prepared by the Williams quinine sulfate procedure is administered to polyneuritic rats the curative dose is equivalent to 10 mg. of acid clay. Thus it is evident that quinine sulfate liberates from the acid clay approximately twice as much vitamin as is extracted by the other procedures. This difference is not due to a potentiating influence of quinine for we have found that the quantity of crystalline vitamin B_1 necessary to cure polyneuritis in rats is practically the same whether administered in aqueous solution of in a solution to which 0.5% of quinine sulfate has been added.

Within the past few months there have appeared several papers reporting on the potency of crystalline vitamin B_1 hydrochloride in terms of international units. Waterman and Ammerman,[5] using a rat growth assay method, found 200 international units per milli-

[4] Smith and Seidell, *U. S. Public Health Reports*, 1936, **51**, 685.

[5] Waterman and Ammerman, *J. Nutrition*, 1935, **10**, 35.

gram; Moll,[6] using both the pigeon day dose method and a rat curative method, reports 500 international units per milligram; Smith, by his rat curative method, finds 333 international units per milligram; B. C. Jansen,[7] using a modified Smith curative method, finds approximately 300 international units per milligram; and Kinnersley and Peters, by their pigeon curative method, interpreted in terms of their catatorulin test report 500 international units per milligram.

Our own findings approximate either 400 international units per milligram or 200 international units per milligram, depending upon whether the untreated international standard clay or its quinine sulfate extract are used for comparison. The larger figure of 400 international units per milligram falls generally within the range reported by others using a curative technic, whereas, the lower figure of 200 international units per milligram is in close agreement with the findings obtained by employing a growth method of assay. This may be interpreted as indicating that only 50% of the vitamin present in the standard adsorbate product is available to the severely depleted polyneuritic rats, whereas the total vitamin effect is obtained on the less severely depleted animals used in a growth test.

The fact that the quinine sulfate extract of the international standard is apparently twice as potent as either the untreated clay or its alkaline extracts indicates that the form in which this substance is administered will largely determine the response. It would seem, therefore, that any estimation of vitamin activity in terms of the present international unit must be weighted in terms of the method of assay employed; before such a comparison is made it should be determined that the vitamin present in both standard and test substance is available to the test animal to the same extent.

Summary. A comparison of different methods of removing vitamin B_1 from the international standard clay shows that the quinine sulfate method yields approximately twice the amount of vitamin that is obtained by alkaline extraction methods.

[6] Moll, *E. Merck Jahresbericht*, 1935, **49**, 56.

[7] Jansen, *Z. fur Vitaminforschung*, 1936, **5**, 254.

9104

The Persistence of Effect of Thevetin.

H. B. Haag and W. A. Pennington.

From the Department of Pharmacology, Medical College of Virginia, Richmond.

Thevetin is a glucoside obtained from the kernels of the "bestill" tree; *Thevetia neriifolia* (Jussieu) family Apocynaceae. Chen and his associates[1, 2, 3] have discussed the bibliography and described the various constituents of these nuts. They have also studied experimentally and clinically the biological potency and characteristic digitalis effect of thevetin.[1-6]

A limited number of experiments concerned with the persistence of effect of thevetin in the cat has been reported by Chen and Chen,[2] the results of which indicate that the physiological effect of thevetin endures only for a relatively short period of time, approaching ouabain in this respect.

In view of recent observations which we have made on the persistence of effect of several digitalis substances in the pigeon,[7] it was of interest to extend our studies to include thevetin for purposes of comparison. About 130 pigeons were employed in the present observations.

The thevetin* was standardized by the intravenous pigeon fatal dose method as described previously.[8] A 1-10,000 thevetin solution was injected into the alar vein of etherized pigeons, 0.5 cc. every 10 minutes. The minimum lethal dose (M.L.D.), *i. e.*, weight of bird under amount of drug, was determined for 24 pigeons. The average M.L.D. was 1.95 mg. per kg. Thevetin was also standardized upon 10 cats according to the technic of Hatcher and Brody[9] and in conformity with experiments of Chen and Chen.[2] The cat unit was 1.0 mg. Chen and Chen[1] originally obtained a slightly higher

1 Chen, K. K., and Chen, A. Ling, *J. Pharm. and Exp. Therap.*, 1933, **48**, 270. (Proceedings).

2 Chen, K. K., and Chen, A. Ling, *J. Pharm. and Exp. Therap.*, 1934, **51**, 23.

3 Chen, K. K., and Chen, A. Ling, *J. Biol. Chem.*, 1934, **105**, 231.

4 Arnold, Harry L., Middleton, William S., and Chen, K. K., *Am. J. Med. Sci.*, 1935, **189**, 193.

5 Middleton, William S., and Chen, K. K., *Am. Heart J.*, 1936, **11**, 75.

6 Noble, Thomas B., and Chen, K. K., *Am. J. Med. Sci.*, 1936, **192**, 639.

7 Haag, H. B., *J. Pharm. and Exp. Therap.*, 1936, **58**, 42.

* We wish to thank Eli Lilly & Company for their kindness in supplying the sample of thevetin used in these studies.

8 Haag, H. B., and Woodley, J. D., *J. Pharm. and Exp. Therap.*, 1934, **51**, 360.

9 Hatcher, R. A., and Brody, L. G., *Am. J. Pharm.*, 1910, **82**, 360.

figure: 1.24 mg., but subsequently,[2] with a more purified product, they found the cat unit to be 0.85 mg. From our studies upon pigeons and cats thevetin appears to be about 1/10 as active as ouabain, U.S.P. XI. Chen and Chen[2] found that purified thevetin was about 1/8 as toxic for frogs, and 1/7 as toxic for cats as ouabain.

In performing the persistence studies, 60% of the average M.L.D. of thevetin was injected intravenously into a series of pigeons, and then at various intervals the amount of thevetin necessary to produce death was ascertained by slow intravenous injection (0.5 cc. of a 1-10,000 solution every 10 minutes). Obviously, then, the difference between this fatal dose and that previously established for normal controls would represent the thevetin effectiveness still remaining. The average value secured from 11 to 20 birds was used for determining the degree of persistence at each time interval studied. As has been noted for other digitalis substances,[7] there were extremely wide individual variations in the degree of continuance of action exhibited.

Following the injection of 60% of the average M.L.D. of thevetin into the 110 birds used for the actual persistence studies 35, or 31.8%, died within several hours. This necessitated a recalculation of the lethal dose for the surviving animals, which was done in the manner previously described,[7] with the result that the "compensated" average M.L.D for these tolerant birds was raised from 1.95 mg. to 2.18 mg. Initial doses of 75% of the average M.L.D.

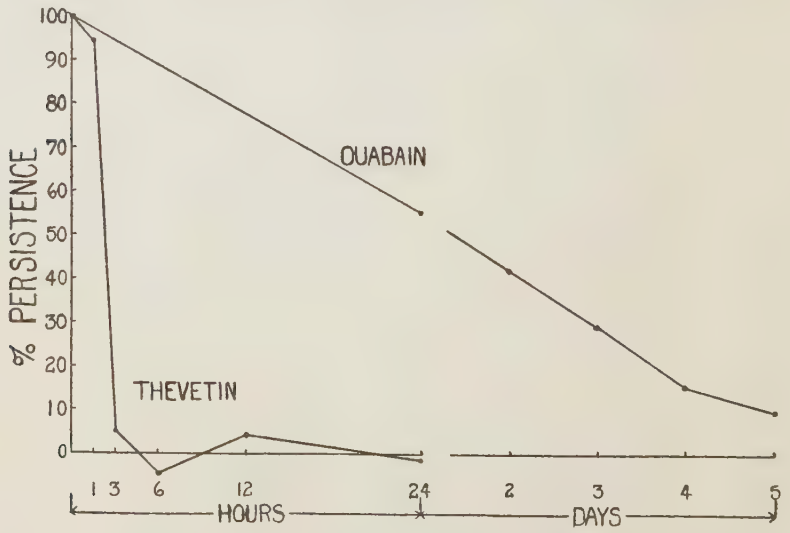

FIG. 1.
Persistence of Action of Thevetin and Ouabain in Pigeons.

resulted in such a high mortality that they could not be used in these studies.

Graph 1 depicts the results obtained in these persistence studies on thevetin as compared with a previously established curve for ouabain.[9] We were astounded by the relative evanescence of the thevetin action, finding practically no persistence of effect in the pigeon after 3 hours. Similar experiments on 8 cats (employing an initial dose of 75% of the average M.L.D.) showed about 15% of its effectiveness remaining after 24 hours. This figure approaches the limit of error for this method of bioassay. These experiments upon pigeons and cats indicate that thevetin is one of the most rapidly eliminated (physiologically) digitaloids yet described.

9105 P

Ureteral Pain as Determined by Faradic Stimulation in Man.

NELSE F. OCKERBLAD AND HJALMAR E. CARLSON. (Introduced by C. J. Weber.)

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In studying the ureteral contractions by the electrical method as described by Paladini,[1] it occurred to us that the localization of ureteral pain could be studied by applying a stimulus to the electrode and having the patient describe the area in which the pain was felt. Experiments of this kind have been extensively done on the pleura, pericardium and peritoneum by Capps[2] and more recently by Boyden and Rigler[3] on the gastrointestinal tract.

Since the electrodes used in the contraction studies were not quite suitable for pain distribution studies, a special electrode with a single metal contact at the tip was manufactured for this purpose by the American Cystoscope Makers, Inc. A Harvard inductorium was used for the source of current with 6 volts on the primary circuit. The inactive electrode was applied to the chest.

The patient was cystoscoped, the ureteral catheter electrode was introduced into the ureter and stimulation was made at various

1 Paladini, A., *Arch. ital. di urologia*, 1934, **11**, 211.

2 Capps, J. A., An Experimental and Clinical Study of Pain in the Pleura, Pericardium and Peritoneum, Macmillan Company, New York, 1932.

3 Boyden, E. A., and Rigler, L., *J. Clin. Invest.*, 1934, **13**, 833.

levels up the ureter. The distance between stimulation points was 5 cm., since it was felt that too frequent stimulation led to indefinite and inaccurate interpretation. After the conclusion of the experiment pyeloureterograms were taken to exclude pathology in the urinary tract.

The results obtained in 8 patients so studied may be briefly summarized as follows:

In the lower one to 2 cm. of the ureter, the pain was referred suprapubically, being almost in the midline and extending upward about 4 cm. Pain into the perineum occurred in some cases but was not constant. Five centimeters from the ureteral orifice the pain was higher and somewhat lateral to the midline. The pain was always below the iliac crest and somewhat medial to McBurney's point. In many cases the patient complained of pain in the inside or outside of the thigh or inside of the leg at this level. In others, pain in the leg was only noticed at somewhat higher levels. From 10 to 20 cm. the pain was referred in practically the same abdominal areas as the lower levels but pain on the inside of the leg often extending to the toes was the rule. At the 25 cm. level the patient complained of pain over the anterior portion of the iliac crest and anterior iliac spines which was also present at the 26 and 27 cm. level. When the interior of the kidney was stimulated the pain was always referred to the back. In one case with a left chronic hydronephrosis and hydroureter no pain response could be elicited, which is in accord with clinical experience in these cases.

9106 P

Excitant Action of Morphine on the Long-surviving Decorticated Cat.*

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Morphine is known to be excitatory to several species of animals. The cat especially gives a violent maniacal response. As the first step in an analysis of this excitant action, the effect of morphine has

* This investigation has been made with the assistance of a grant from the Committee on Therapeutic Research, Council on Pharmacy and Chemistry, American Medical Association.

† Department of Pharmacology, Western Reserve University, Cleveland, Ohio.

been studied on the long-surviving cat after bilateral cerebral decortication. Up to the present time 4 such animals have been prepared, and the response of each to morphine has been observed on from one to 3 separate occasions.

Operation was done aseptically in 2 stages at least 2 weeks apart under sodium amytal anesthesia (50 mg. per kg., intraperitoneally), using a fronto-parietal approach. The cortex of each cerebral hemisphere was peeled off with blunt spatula, sparing the olfactory cortex as much as possible.

Anatomical Study. Gross examination of the formalin-hardened brains disclosed absence or probable degeneration of the entire cortex except for parts of the olfactory system, and considerable destruction of the striate nuclei. The thalami were largely intact. Histological study of the brains is now in progress. These cats were, therefore, essentially *Thalamic Preparations.*

Physiological Activity. The behavior of the animals will be discussed in detail in a later publication. The activity varied considerably in the different animals. Cats No. 2 and 3 showed very little spontaneous walking, but stood in one position most of the time, whereas the other 2 cats walked nearly continuously. All of them were attracted by the smell of food, but were unable to eat sufficient to maintain body weight, except cat No. 4, which drank milk spontaneously and required no supplementary feeding. The other cats were fed milk daily by stomach tube (which was done merely as a time-saving measure since they were all able to swallow milk when it was put in the mouth). The animals which walked had a high-stepping, spastic gait. The placing and hopping reactions and the ability to correct imposed abnormal postures were tested‡ in the last 3 cats and were all absent.

Results. All 4 cats, 2 to 11 weeks after total decortication, responded to morphine by a kind of excitement which was somewhat modified from, yet fairly characteristic of the effect produced in intact cats. The difference could largely be attributed to the absence of the postural and related reflexes. Thus the intact morphinized cat (10 to 20 mg. per kg., subcutaneously) typically shows bursts of violent maniacal charges from one side of the cage to the other. These alternate with slightly longer periods of relative inactivity during which the cat sits up with alert and roving head and eyes. The morphinized decorticated cat also shows alertness of head

‡ The author wishes to acknowledge his indebtedness to Dr. Clyde S. Marshall of the Department of Neuroanatomy, Yale University, for his valuable assistance with these tests.

and eyes and periodic bursts of activity. But while resting it lies with stiffly extended legs, and the periods of activity are characterized by generalized muscle spasms which sometimes propel the animal across the floor. If set on its feet, the cat tends to take several quick, spastic, trot-like steps before it falls. This is the phase of the activity which most closely resembles the morphine mania seen in intact cats. It differs mainly (1) by the inability to assume or maintain an upright position, (2) by pronounced extensor spasm of the legs and (3) by quicker onset of fatigue.

Both intact and decorticated cats a few hours after morphine administration show a heightened "startle" reflex to noise (clapping of hands) and to tapping the animal. This effect is exaggerated in the decorticated cats and closely resembles the tetanus of early strychnine poisoning. Two of the decorticated animals in this condition were given a half-anesthetic dose of sodium amytal (25 mg. per kg., intraperitoneal) which uncovered vigorous running movements not seen in intact cats similarly treated.

Summary. The long-surviving cat after bilateral cerebral decortication with degenerative changes in the striate nuclei responds to morphine with an excitement which is quite similar to the effect on intact cats, but with certain modifications described in the text. The excitant action of morphine must, therefore, be mediated by subcortical centers.

9107

A Method for Determining Blood Volume in Rats.

J. Q. GRIFFITH, JR.,* AND R. CAMPBELL. (Introduced by J. H. Austin.)

From the Robinette Foundation, the Medical Clinic, and the Pepper Laboratory of the Hospital of the University of Pennsylvania.

Methods for determining the blood volume of small animals are available, but all have one of two objections: (1) they require so much blood as to affect the subsequent blood volume of the animal, or may even cause its death; (2) they require the laborious preparation of standards consisting of dye-containing serum in uniform capillary tubes. Such standards are then presumed to be permanent.

Our method requires but 50 cmm. of blood, and the standard can

* Heckscher Fellow in Medicine.

be set up for each period of work in a few minutes. Moreover, the blood volume is determined directly, and not calculated from the serum volume by means of the hematocrit reading.

Adult albino rats have been used. Three mg. of nembutal per 100 gm. of body weight are injected intraperitoneally. As soon as the animal is sufficiently quiet, the jugular vein is exposed at the base of the neck by an incision parallel to its course. Then 0.3 cc. of 5% vital red is injected into the dilated bulb. The manner of injection is important. A one cc. tuberculin syringe fitted with a fine hypodermic needle is used. Exactly 0.3 cc. of the dye is drawn into the syringe, reading the mark against a strong light. The needle, pointing toward the rat's head, is introduced into the upper border of the pectoral muscle, grazes the upper edge of the clavicle and enters the vein through the muscle. The point is carried onward until it is visible in the jugular bulb. It is moved up and down to be sure it has not picked up the vein wall, after which the dye is injected under inspection. We wait 20 to 25 seconds while the vein clears of dye. Then the needle is quickly withdrawn and pressure exerted momentarily over the muscle. Leakage does not occur. This is the only method we have been able to find or to devise by which small intravenous injections can be given quantitatively in the rat.

After 3½ minutes the tail is immersed in water at approximately 45°C. for one minute. Then, 4½ minutes from the time of the injection, a vein in the tail is punctured, and 50 cmm. of blood is withdrawn into a graduated pipette. The puncture wound is sealed with collodion. The 50 cmm. of blood are placed in 2.95 cc. of physiologic saline (3 cc. from which 50 cmm. have been removed) and centrifuged. The supernatant fluid is pink.

The standard is prepared by adding 0.3 cc. of 5% vital red to 4 cc. of physiologic saline, the resulting volume being 4.3 cc. This is the primary standard. Standard A is made by adding 50 cmm. of this to 5.95 cc. of physiologic saline (6 cc. from which 50 cmm. have been removed). Standard B is made by adding 100 cmm. of the primary standard to 5.9 cc. of physiologic saline.

We use a microcolorimeter† requiring 2 cc. of fluid in each cup. The standard cup is connected by a sidearm to a 1 cc. tuberculin syringe graduated to 0.01 cc. The volume of the standard in the standard cup (VS) is therefore 2.00 minus the amount in the syringe, read to the second decimal. In an actual determination

† The colorimeter used is the Vim-Sheftel Microcolorimeter, made by the MacGregor Instrument Co., Needham, Mass.

2 cc. of the unknown are introduced into the cup for the unknown. This is the pink supernatant fluid obtained after centrifugalization. Two cc. of standard A or B are introduced into the standard cup, the one being selected which appears slightly darker than the unknown. Standard is then withdrawn into the syringe until the colors match. The formulas are: for standard A, blood volume = 17.2/VS; for standard B, blood volume = 8.6/VS.

Results. The average blood volume in 20 rats was 4.3 cc. per 100 gm. of body weight, varying from 4.1 to 5.3 cc. The smaller animals have the larger blood volumes in terms of body weight. Only 3 animals in this series had body weights below 160 gm., and only these had blood volumes of 5 cc. or more per 100 gm. of body weight.

If the course of disappearance of dye is followed at 15 minute intervals after injection, it is found that a gradual loss occurs reaching 11 to 12% in one hour.

As a control, 4 rats were injected by the same technique with 0.3 cc. of 0.4% vital red, and exsanguinated at the end of 4½ minutes. One cc. or more of serum was obtained and this, undiluted, was read in the colorimeter against a standard set up in normal serum. These rats had an average blood volume of 4.8 cc. per 100 gm. body weight, varying from 4.4 to 5.2.

Method for Repeated Determinations. This method is applicable when dye from a previous injection is still present in the blood stream. The only change is in the making up of standards A and B. It is estimated from the size of the animal which standard, A or B, will be required. If there is doubt, both must be made up. Take 2.95 cc. of the selected standard and add to it 50 cmm. of blood taken just before the second dye injection. Centrifuge, and place 2 cc. of the supernatant fluid in the standard cup. The remainder of the procedure and calculation is unchanged.

Our results agree with those of Cutting and Cutter,[1] who determined serum volume but who give hematocrit values in their protocols from which blood volume can be calculated. They disagree with the results of Went and Drinker[2] who, in 7 animals, found an average blood volume of 7.4 cc. per 100 gm. of body weight with a variation of 6.9 to 7.9.

1 Cutting, W. C., and Cutter, R. D., *Am. J. Physiol.*, 1935, **113**, 150.

2 Went, S., and Drinker, C. K., *Am. J. Physiol.*, 1929, **88**, 468.

9108

Effects of Cortico-Adrenal Extract on the Estrus Cycle of Hypophysectomized Rats.*

E. L. Corey.

From the Physiological Laboratory, University of Virginia Medical School.

An adreno-hypophyseal interrelationship has been strongly indicated by numerous lines of evidence. The experiments of Smith,[1] Richter and Wislocki,[2] Atwell,[3] and Cutuly[4] have shown that extensive atrophy of the adrenal cortex follows complete hypophysectomy in the rat. Collip and his co-workers[5] have established the elaboration of an adrenotropic hormone by the pituitary, and alterations in carbohydrate metabolism have been shown to follow the ablation of either or both glands.[6–12] Long[13] has suggested that the hypophysis may control sugar formation from protein through the mediation of the adrenal. Furthermore, Swingle, *et al.*,[14] report life-prolonging effects from the administration of hypophyseal and anterior-pituitary-like extracts to adrenalectomized cats and dogs. The work of Kitagawa,[15] Wyman,[16] Martin,[17] Martin and Kroc,[18] and Corey and Britton[19] on the cessation of estrus following

* Aided by a grant from the Rockefeller Foundation.

1 Smith, P. E., *Am. J. Anat.*, 1930, **45**, 205.

2 Richter, C. P., and Wislocki, G. P., *Am. J. Physiol.*, 1930, **95**, 481.

3 Atwell, W. J., *Endocrinology*, 1932, **16**, 639.

4 Cutuly, E., *Anat. Rec.*, 1937, **66**, 119.

5 Collip, J. B., Anderson, E. M., and Thomson, D. L., *Lancet*, 1933, **2**, 347.

6 Houssay, B. A., Biasotti, A., and Rietti, C. T., *Compt. rend. Soc. de Biol.*, 1932, **111**, 479.

7 Smith, P. E., Dotti, L., Tyndale, H. H., and Engle, E. T., Proc. Soc. Exp. Biol. and Med., 1936, **34**, 247.

8 Phillips, R. A., and Robb, P., *Am. J. Physiol.*, 1934, **109**, 82.

9 Fisher, R. E., and Pencharz, R. I., Proc. Soc. Exp. Biol. and Med., 1936, **34**, 106.

10 Chaikoff, I. L., Reichert, F. L., Larson, P. S., and Mather, M. E., *Am. J. Physiol.*, 1935, **112**, 493.

11 Britton, S. W., and Silvette, H., *ibid.*, 1934, **107**, 190.

12 Corey, E. L., and Britton, S. W., *ibid.*, 1937, **118**, 15.

13 Long, C. N. H., *Ann. Int. Med.*, 1935, **9**, 166.

14 Swingle, W. W., Parkins, W. M., Taylor, A. R., and Morrell, J. A., Proc. Soc. Exp. Biol. and Med., 1936, **34**, 94.

15 Kitagawa, S. N., *Fujiuko. Gaz. Z.* 22, 1927 (Biol. Abstracts).

16 Wyman, L. C., *Am. J. Physiol.*, 1928, **85**, 414.

17 Martin, S. J., *ibid.*, 1932, **100**, 180.

18 Martin, S. J., and Kroc, R. L., *ibid.*, 1933, **105**, 71.

19 Corey, E. L., and Britton, S. W., Proc. Soc. Exp. Biol. and Med., 1933, **30**, 592.

adrenalectomy and the restorative effects of cortico-adrenal extracts points in the direction of such an interrelationship.

The present study was designed to determine whether cortico-adrenal extracts possess any estrus-restoring properties when administered to hypophysectomized rats. Three dozen female rats were obtained from the colony of the Wistar Institute,† of which 24 were of pure "Wistar" stock and 12 of the "red-eyed-yellow" Castle strain. All animals were between 5 and 7 months of age. The periodicity of the cycles of all rats was established by the vaginal smear method of Long and Evans[20] for 2 to 6 weeks prior to operation. Twenty-six animals were completely hypophysectomized‡ and a total of 34 individual tests were made. No test animal showed an estrous type of smear for more than 2 days following operation, and all reacted positively to the injection of 5 units of "Progynon-B" (estradiol benzoate). The test dosage of cortico-adrenal extract (made by a modified Swingle-Pfiffner technique[21]) was varied from 3 to 10 cc. per day. In the case of a "positive" response, injections were immediately discontinued and subsequent vaginal smears studied to check the possibility of an incomplete hypophysectomy. Otherwise the tests consisted in daily injections of increasing amounts of extract over a period of 7 days.

Of the 34 tests made, 28 were definitely negative and 6 were classified as "positive" (heavy smear—cornified cells only). One animal which showed a negative reaction to the extract alone exhibited the estrous type of smear on 2 successive days following 2 injections of one mg. of thyroxin plus 5 cc. of extract. Continued treatment yielded only negative results and the animal died in diestrus 6 days after beginning the injections.

In another case treatment was begun 35 days after operation and continued for 3 days. A smear of the estrous type was obtained on the day after the first injection, persisting for 4 days. The rat died 24 days later, having shown no subsequent smears indicative of estrus.

Four rats which received extract on the day following operation showed one "positive" smear four days later with no further indications of estrus until death (at an average of 14 days after ces-

† Obtained through the kindness of Dr. M. J. Greenman.

20 Long, J. S., and Evans, H. M., *Univ. Calif. Memoirs,* 1922.

‡Completeness of hypophysectomy was judged in all cases by the daily vaginal smear record and by gross autopsy findings at the termination of the experiments (examination of the sella; presence of uterine and adrenal atrophy; loss of weight).

21 Britton, S. W., and Silvette, H., *Am. J. Physiol.,* 1931, **99**, 15.

sation of treatment). In none of the remaining tests (28) did indications of estrus appear, although some animals were subjected to repeated courses of injection.

From the quantitative standpoint, therefore, the results were negative, and similar to those previously obtained[22] in tests of adrenal extracts on ovariectomized rats. In the latter experiments the estrous type of smear appeared in about 30% of cases, although the results were statistically negative. It is concluded that corticoadrenal extracts of known life-maintaining potency possess no estrogenic value when administered to completely hypophysectomized rats.

9109 P

Non-specificity of Orchid Mycorrhizal Fungi.

John T. Curtis. (Introduced by B. M. Duggar.)
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While isolating the mycorrhizal fungi from various species of native Wisconsin orchids, several features were noted which are at variance with the usually reported results of European investigators concerning the specificity of mycorrhizal infections. Thirty-three isolations have been made from temperate species, of which at least 16 are morphologically different. All of the strains* possess typical Rhizoctonia "spore-forms" as described by Bernard[1] for fungi from tropical and European orchids. One also has a perfect stage with basidia, but its exact taxonomic status has not yet been determined.

There is an apparent correlation between ecological habitat and fungus type, rather than between orchid species and fungus. For example, *Habenaria leucophaea* from a tamarack-sphagnum bog was infected with a different strain of Rhizoctonia from that found in the same orchid growing in an open prairie. The two fungus strains differed markedly in spore size, growth characteristics and physiological properties. On the other hand, 4 other orchid species from the above sphagnum bog, *Habenaria dilatata, H. hyperborea,*

22 Corey, E. L., and Britton, S. W., *ibid.*, 1934, **107**, 207.

* The word "strains" is here used to denote cultures differing markedly in both morphological and physiological characteristics.

1 Bernard, Noel, *Ann. Sci. Nat. Bot.*, 1909, **9**, 1.

H. lacera, and *Pogonia ophioglossoides,* were all infected with a fungus strain morphologically identical with that from the bog *H. leucophaea.* In another instance, 3 widely separated orchid species, *Goodyera pubescens, Habenaria psycodes,* and *Liparis liliifolia,* growing in and on the sides of a rocky pine-covered ravine, all contained the same fungus.

The extent of this non-specific infection is seen in the number of distinct strains found in the same orchid species from diverse localities in Wisconsin. Thus 3 Rhizoctonia strains were isolated from *Spiranthes cernua,* 3 from *Pogonia ophioglossoides,* and 4 from *Habenaria leucophaea.* Perhaps the most significant finding was the growth of 2 entirely different strains of Rhizoctonia from opposite ends of the same root piece of *Habenaria leucophaea* on the isolation plate. In one the diameter of the hyphae and "spore-cells" was 4 times that of the other, while the form and number of the "spore-cells" was also distinct. In addition to these cases where morphologically unlike fungi were found in the same orchid, strains exhibiting marked physiologic differences also occurred. Thus 2 microscopically similar strains would often possess distinct carbohydrate and nitrogen requirements, and would produce different Eh and pH changes in the medium.

These observations appear to conflict with the assumptions of Bernard[1] and Burgeff,[2] who held that each orchid species or group of related species contained its own specific mycorrhizal fungus, which was intimately concerned with the physiological well-being of the plants and with the germination of the seeds. These observations seem rather to uphold the theory that the orchids may be infected by any mycorrhizal Rhizoctonia present in the surrounding soil, and that the character of the fungus is determined more by the nature of the ecological habitat than by the species of the orchid.

I wish to express my appreciation to Dr. B. M. Duggar, under whom this investigation was carried out, and to Dr. E. M. Gilbert, for his continued help and advice.

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[2] Burgeff, Hans, *Samenkeimung der Orchideen,* Jena, 1936.

9110 P

Breathing of Amniotic Fluid as a Normal Function of Fetal Respiration.

Franklin F. Snyder and Morris Rosenfeld.

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The demonstration of rhythmical respiratory movements of the fetus long before term raises the question whether or not amniotic fluid normally enters the lungs before birth as a result of these movements.[1, 2, 3] In order to determine the existence of a tidal flow of amniotic fluid in the respiratory tract, India ink was added to the amniotic fluid. In a typical experiment the uterus of a rabbit at term, *i. e.*, 32 days, was exposed by laparotomy carried out beneath the surface of a bath of Ringer solution at 37°. General anesthesia was avoided by section of the spinal cord in the lumbar region, and inhibition of uterine contractions was obtained by injection of one cc. (100 rat units) of Antuitrin S (Parke, Davis & Co.) on the 25th day of pregnancy. The head of a fetus was distinguished readily through the transparent uterine wall. One cc. of 50% India ink was injected within the amniotic sac in the dorsal neck region between the ears. After intervals ranging from one to 60 minutes the trachea was closed by clamping the neck of the fetus. The lungs were examined and fixed in formalin before removal of the clamp.

Comparison was made of the lungs of fetuses which had been showing respiratory movements with those of litter-mates in which breathing had been suppressed by injection of pentobarbital sodium. In breathing fetuses, the lungs were blackened, while in contrast in apneic fetuses the lungs were normal. Microscopical examination showed carbon particles in the alveoli of the lungs of the former but not the latter.

In litter-mates which were breathing at different rates, it was found that the lungs were darker in those which showed the greater respiratory activity. For example, in a fetus breathing at a rate of 96 per min. for 5 min. after addition of one cc. of ink to the amniotic fluid, the lungs were much darker than those of a litter-mate removed after 16 minutes of breathing ink-stained fluid

1 Snyder, F. F., and Rosenfeld, Morris, *Am. J. Physiol.*, 1937, in press.

2 Wislocki, G. B., *Contrib. to Embryol., Carnegie Inst. of Washington*, 1920, **11**, 47.

3 Klemperer, H. H., *Arch. f. Gynak.*, 1933, **154**, 108.

at a rate of 10 per minute. The magnitude of the exchange afforded by fetal respiratory movements was illustrated by lungs removed within the first minute following introduction of ink within the amniotic sac. Darkening of the lung was clearly evident.

Observations are based upon 25 fetuses obtained from 7 rabbits. Fourteen fetuses were breathing at the time of injection while 11 were apneic.

In conclusion, it is clear that amniotic fluid normally occupies the alveoli of the fetal lung. The spontaneous respiratory movements of the fetus are responsible for a tidal flow in the respiratory tract. There is evidence that intrauterine respiration is of functional significance in the development of a normal lung, aiding in dilatation of the alveoli and elastic walls of the future air passages.

9111

Choline-Esterase Activity of Human Sera, with Special Reference to Hyperthyroidism.*

WILLIAM ANTOPOL, LESTER TUCHMAN AND ARTHUR SCHIFRIN.
(Introduced by Harry Sobotka.)

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Until 1932, the rate of enzymatic destruction of acetylcholine was determined biologically. In that year, Stedman[1] and his coworkers described a chemical method for assaying the acetylcholine splitting enzyme in the blood by titrating the acid liberated from the hydrolysis of acetylcholine at a constant pH. This esterase also acted upon butyrylcholine. In 1933, they found that the cholinesterase of human serum was relatively high and showed considerable individual variation.[2] This activity also varied widely in the different species.

In 1933, Ammon[3] employed a gasometric method by which he determined the amount of carbon dioxide liberated from a carbonate solution by the acid formed from the hydrolysis of the acetylcholine substrate. The carbon dioxide was measured in the Bar-

* Aided by a grant from the Committee on Scientific Research of the American Medical Association.

1 Stedman, E., Stedman, E., and Easson, L. H., *Bioch. J.*, 1932, **26**, 2056.

2 Stedman, E., Stedman, E., and White, A. C., *Bioch. J.*, 1933, **27**, 1055.

3 Ammon, R., *Pflügers Arch. f. d. ges. Physiol.*, 1933, **233**, 486.

croft-Warburg respirometer, and the amount formed served as an index of the acetylcholine splitting power. In the meantime, Stedman independently devised a method, utilizing the Barcroft differential apparatus, which was based upon the same principle. The advantages of these methods far surpassed the titration procedure since red blood cells and colored solutions could be tested, whereas the pigments interfered with the older procedure.

In 1935, Ammon and Voss[4] showed that human sera had a weaker splitting activity than whole blood. They also confirmed Stedman's 1932 findings of marked variations of human blood, and in addition, found that individuals had constant splitting powers over a period of several weeks.

In 1936,[5] Von Verebely, returning to a biological method, confirmed Ammon's findings.

We have made upwards of 500 determinations on unselected hospitalized adults and 60 selected, apparently normal individuals of essentially the same age group as the pathological cases. We used Ammon's procedure,[3] except that the reaction was permitted to continue over a 2-hour period in the water bath at 30°C. and that the spontaneous hydrolysis of acetylcholine was determined with each experiment and this result subtracted from the total figure.† The first hundred determinations were performed in duplicate and the differences between the duplicates were found to be insignificant. In many of these, eserine in high dilutions ($1:10^8$) completely inhibited the enzymatic scission. The control group consisted of professional blood donors and members of the hospital personnel. Sixty consecutive subjects in this so-called normal group revealed an average reading of 67.6 mm^3., with all cases except 7 falling between 44 and 80 mm^3. The exceptions were 31, 81, 82, 83, 86, 95, and 99. (Table I.) It was not possible to determine the causes for these extreme figures since many of this group were not available for further study. Many of the controls were repeated over a period of weeks and the activity of the sera obtained from the same individual at different times remained virtually constant. A number of sera in this group obtained in the fasting state and after meals revealed but slight variation. Almost all of the specimens in this series, however, were obtained in the fasting state. In one case, blood was taken at 4-hour intervals for a period of 24

4 Ammon, R., and Voss, G., *Pflügers Arch. f. d. ges. Physiol.*, 1935, **235**, 393.

5 Von Verebély, T., Jr., *Kl. Wchnschr.*, 1936, **15**, 11.

† We have found that the spontaneous hydrolysis of the acetylcholine solutions, which were made up separately for each experiment, varied despite the fact that the salt was obtained from the same bottle.

TABLE I.
Control Group.

31	44	50	60	70	80	95
	47	52	60	71	81	99
	47	53	60	71	82	
		53	61	71	83	
		55	62	71	86	
		56	63	71		
		57	63	71		
			63	72		
			64	73		
			64	73		
			64	74		
			64	74		
			65	75		
			65	75		
			65	75		
			67	75		
			67	76		
			69	76		
			69	77		
			69	78		
				78		
				79		

Choline esterase activity of serum expressed in mm³. CO_2 developed in 2 hours at 30° C.

TABLE II.
Cases of Hyperthyroidism.

Untreated (22 Cases)	Treated (13 Cases)
117	100
109	85
109	85
104	83
103	77
102	74
99	73
98	70
97	68
96	66
90	62
89	58
87	49
87	
87	
81	
81	
80	
76	
71	
62	
62	
Average 90 mm³.	73 mm³.

Choline esterase activity of serum expressed in mm³. CO_2 developed in 2 hours at 30° C.

hours and the figures varied from 54 to 62 mm³., the highest figure being obtained at 7:00 A. M. and 10:00 A. M., and the lowest at

10:00 P. M. We could not obtain blood during sleep because a subject was not available into whose vein we could tie a cannula.

In the hospitalized cases we found the variations in the hydrolizing effect to be much more pronounced. The figures varied from 8 mm^3. of carbon dioxide to 163 mm^3. of carbon dioxide liberated in a period of 2 hours. The average of these readings was 62 mm^3. All of these specimens were obtained in the fasting state. The disease groups fell within broad but definite ranges. Thus, the group consisting of 35 unselected clinical cases of hyperthyroidism averaged 84 mm^3. Of these, in 22 non-treated cases, the average was 90 mm^3., while the other 13 which had been treated either by lugolization or operation, showed an average of 73 mm^3. (Table II.) The treated case with 100 mm^3. was an instance in which symptoms recurred 2 years after thyroidectomy. A case of nephrosis, not included in this series, which had been receiving thyroid therapy split 129 mm^3. No untreated case of nephrosis was available for comparison.

The recognition of the muscarine action of acetylcholine by Dale, the demonstration by Loewi of a substance formed as a result of vagus stimulation, the so-called "Vagusstoff", which is responsible for transmission of the parasympathetic impulse and which is a choline ester closely resembling acetylcholine, and in its effects, virtually identical with acetylcholine, the evidence of destruction of this substance by an esterase which is physiologically indigenous throughout the body, in conjunction with the findings herein recorded, lend support to the belief that the choline esterase activity may be one of the elements related to the so-called vagotonicity or sympathicotonicity of an individual. Inferentially, this choline-esterase activity may be a factor which is inversely related to the vagotonicity and parallels the sympathicotonicity of the individual. The fundamental basis for this hypothesis centers about the individual variations in the potency or amount of choline-esterase, determining the rate of destruction of the vagus substance. In the so-called sympathicotonic person there may be an increased amount of or more potent enzyme; in vagotonic individuals, there may be less enzyme or a weaker enzyme. The former type would obviously destroy the vagus substance rapidly, influencing the ensuing autonomic equilibrium in such a manner that the sympathetic factors are comparatively increased. The latter type may destroy it more tardily, thus sparing the vagus substance which remains relatively unaltered and capable of activity over a longer period of time with a result simulating a summation effect in which the parasympathetic factors predominate.

Although we have not sufficient data on diabetes mellitus, it appears that the non-treated severe diabetic figures approximate or surpass the upper limit of normal, dovetailing with the thyroid group, whereas the treated cases are grouped in the lower levels of the normal range. Some of the hypertensive cases also exceed the higher normal figures. On the other hand, many of the sera obtained from patients with jaundice, cirrhosis of the liver, hepatitis, anemia, arthritis, rheumatic fever, and hyperpyrexia reveal pronouncedly depressed values. Further studies are now being conducted to clarify the significance of these results.

Summary. The acetylcholine esterase activity of the blood serum was determined in 500 individuals including normal and pathological cases. A modification of the Ammon gasometric method was utilized. The acetylcholine esterase was relatively high in cases of untreated hyperthyroidism.

The acetylcholine esterase activity may be one of the elements related to the so-called sympathicotonicity or vagotonicity of an individual.

9112 P

Hepatic Excretion in Man of the Various Bile Acids Following Their Oral Administrations.

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It is well known that animals normally excrete in their bile the various bile acids specific to their species. When foreign bile acids are administered by mouth they are readily absorbed and excreted by the liver. Weiss[1] as well as Prevost and Binet[2] showed that orally administered, sodium glycocholate, ordinarily absent in dog's bile, is excreted in the bile. Jenke[3] found an excess of free cholic acid in the bile of a dog after feeding that acid in large amounts.

Human bile contains principally a mixture of cholic and desoxycholic acid in approximately equal proportions; about four-fifths of

* Ralph Colp Fellow in Physiology.

1 Weiss, A., *Centralbl. f. die Med. Wiss.*, 1885, **23**, 121.

2 Prevost, J. L., and Binet, Paul, *Comp. rend. acad. de. sc.*, Paris, 1888, **106**, 1690.

3 Jenke, M., *Arch. f. exp. Path. u. Pharmakol.*, 1928, **130**, 280.

these acids are normally combined with taurine and glycine to form the conjugated bile acids.[4] It was thought that the administration of various pure bile acids to a human might shed some light on the mechanism of their intestinal absorption and of their excretion by the liver cell.

Pure cholic and desoxycholic acids were fed in large amounts to a patient with a complete common bile duct fistula resulting from pancreatic obstruction. After removing a sample for analysis the total 24-hour excretion of bile was refed the next day in 3 portions. The extra bile acids administered were dissolved in a small quantity of water and added to the bile. A recently developed method[5] was used to analyze the daily excretion of bile for bile acids conjugated with taurine and with glycine, for cholic acid, for desoxycholic acid and for free bile acids.

During the first experimental period (Mar. 9-12), the addition of 3, 6 and 6 gm. of pure cholic acid on consecutive days, raised the percentage composition of the bile from 1.71% to 2.09%, the increase being mainly due to an increased percentage of cholic acid. There was a parallel increase in conjugation of the acids. This increase, however, was wholly in the form of glycine conjugated bile acids. The total excretion rose from 10 gm. to a maximum of 14.2 gm. The administration of cholic acid resulted mostly in the increased excretion of cholic acid, but it was noted that the amount of desoxycholic acid was also increased.

During the second experimental period very large amounts of both cholic acid and desoxycholic acids were fed to determine the maximum excretory power of the liver in the presence of an excessive intake. No untoward effects were noted by the patient. After a 3-day control period, a total of 40 gm. of pure desoxycholic acid was added to the bile intake of the next 3 days. The total bile acids rose from 1.1% to 1.79%, while the cholic acid fell from 0.4% to 0.13%. The rise therefore resulted from an increased excretion of desoxycholic acid. Conjugation kept pace with increased output of desoxycholic acid. The increase was entirely in the form of glycine conjugation. Two days after the cessation of desoxycholic acid administration, the total bile acid concentration was back to 1.08%; the cholic acid, however, was abnormally high, being 0.70%.

On the administration of 45 gm. of cholic acid during the next 3 days, the cholic acid rose to a maximum of about 1% (similar to

[4] Colp, R., and Doubilet, H., *Arch. Surg.*, 1936, **33**, 913.

[5] Doubilet, H., *J. Biol. Chem.*, 1936, **114**, 289.

that in the first experimental period). The desoxycholic acid percentage, however, rose to a surprisingly high figure, the maximum concentration of total bile acids reaching 2.83%. During that day the liver excreted 11.78 gm. of bile acids. The conjugated bile acids did not increase proportionately with the increase in total bile acids. The volume of bile rose from 390 cc. to a maximum of 690 cc. The bile acid output returned to the control level 5 days after the cessation of cholic acid administration.

Summary. In the patient studied the oral administration of cholic acid was more effective than that of desoxycholic acid in raising the concentration and total output of bile acids in the hepatic bile.

9113 P

An Excretory Test for Vitamin C Deficiency and Subnutrition.

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The excretion of vitamin C in the urine after an intravenous injection of 100 mg. of ascorbic acid was followed in a group of 12 normal subjects on diets adequate in vitamin C, in 3 normal subjects on diets low in vitamin C and in 13 cases of scurvy. The age of the normal subjects varied from 20 to 25 years. The subjects on the diet low in vitamin C were of the same age group. In the patients with scurvy 5 were under 40 years of age and 5 over 60 years of age. The symptoms of scurvy in these patients consisted in changes in the gums, present in all the patients and evidenced by ulceration, hemorrhage into the gums and piling up of the gums. Massive subcutaneous hemorrhage was present in 3 cases and petechiae over the legs and arms in 6 cases. The duration of the deficient diet was 3 months in 4 cases, 12 months in 5 cases and over 12 months in the rest of the patients.

The procedure for the test was as follows: In each group the excretion of vitamin C was determined for a 3-hour period and also for the following 21-hour period, prior to the administration of any vitamin C. The following day, after emptying the bladder, an injection of 100 mg. of ascorbic acid (Merck and Company) was given intravenously to the subject and the urine was again collected for 3 hours and for the following 21 hours. Vitamin C was deter-

TABLE I.

Mg. of Vitamin C Excreted in 3 and 21 Hours on Diet and Following Injection of 100 mg. of Ascorbic Acid in Normal, Subnutrition and Scorbutic Subjects.

Normals				Subnutrition Cases				Cases with Scurvy			
Mg. Excreted on Diet —Hr.—		Mg. Excreted After Test Dose —Hr.—		Mg. Excreted on Diet —Hr.—		Mg. Excreted After Test Dose —Hr.—		Mg. Excreted on Admission —Hr.—		Mg. Excreted After Test Dose —Hr.—	
3	21	3	21	3	21	3	21	3	21	3	21
25.4	180.7	52.7	125.8	1.4	9.5	11.5	4.8	3.3	—	3.6	—
9.0	53.2	24.2	87.1	2.2	11.7	5.6	1.6	2.2	—	1.3	4.7
16.8	81.6	81.8	73.8	2.3	9.5	14.2	9.1	Not done		7.9*	—
21.2	44.7	67.0	89.3					2.1	8.2	3.9	12.9
8.5	109.0	40.5	104.0					No Vitamin C Titrable			
6.4	31.1	32.0	34.6					2.0	6.2	Tr.	0
10.9	36.1	57.8	65.7					0.7	1.5	0.3	2.8
12.9	30.1	44.6	49.3					0.3	0	1.0	0
4.9	39.7	43.5	25.1					0.2	20.9†	9.7†	23.9
4.5	58.9	47.8	53.5					2.0	17.2‡	4.7	14.1
12.1	51.9	37.6	47.9					0	0.75	1.8	21.5
10.9	86.9	34.4	80.9					0	0	0.4	1.5
								0.69	5.2	2.3	6.6

*Clinic patient had been taking some orange juice for week prior to test.
†Clinic patient had been taking orange juice for one week.
‡Patient received 100 mg. Cevitamic Acid after the 3-hour excretion.

mined by the method of Birch, Harris and Ray,[1] which consists of titrating the urine against a standardized solution of 2:6 dichlorophenolindophenol. Precautions were taken for the preservation of the vitamin by adding glacial acetic acid, 10% by volume to the specimens. The overnight specimens were kept in dark bottles in the icebox. All the urines passed during the day were titrated immediately.

Table I shows the results in the normal, sub-nutrition and scurvy subjects. In the normal group, on diet alone the urinary excretion of vitamin C in 3 hours varied from 4.5 to 25 mg., in 21 hours from 31 to 180 mg., with an average excretion of 12 mg. for 3 hours and 67 mg. for the following 21 hours. Following the intravenous injection of 100 mg. of ascorbic acid, the excretion rose in all the normal subjects. The variations were from 25 to 82 mg. in 3 hours, and in the following 21 hours from 25 to 126 mg. The averages were 47 mg. for 3 hours and 69 mg. for 21 hours.

In the subnutrition group on diet alone the 3-hour excretion averaged 2 mg., the 21-hour excretion 10 mg. Following an intravenous test dose of 100 mg. of ascorbic acid the average 3-hour excretion was 11 mg. The average 21-hour excretion was 5 mg.

In the patients with scurvy the excretion in 3 hours on diet alone averaged 1.5 mg. and the excretion in 3 hours following an intravenous test dose of 100 mg. of ascorbic acid averaged 2.6 mg. In some cases no vitamin C was titratable in the urine and in 2 cases (22 and 28) the excretion rose to 8 and 9 mg. in 3 hours, but both of these cases had been taking orange juice for one week on the advice of a physician.

Summary. 1. The 3-hour urinary excretion of vitamin C before and after an intravenous test dose of 100 mg. of ascorbic acid was studied in a group of 12 normal adults on their usual diet which was adequate in vitamin C, in 3 normal adults on diets low in vitamin C and in 13 cases of scurvy. 2. The 3-hour and 21-hour excretion was studied in the same group after an intravenous test dose of 100 mg. of ascorbic acid. 3. Following the test dose the normal subjects excreted an average of more than 40% of the injected vitamin within 3 hours; the subnutrition cases an average of 11% and the scurvy group an average of 2.6%. 4. These observations support the fact that the 3-hour urinary excretion of vitamin C following an intravenous dose of 100 mg. of ascorbic acid will serve as an index of vitamin C deficiency or subnutrition.

[1] Birch, T. W., Harris, L. J., and Ray, S. N., *Biochem. J.*, 1933, **27**1, 590.

9114

Bactericidal Properties of Acrolein.*

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Walker, Lindegren, and Bachmann,[1] Walton, Herbold, and Lindegren,[2] McKnight and Lindegren,[3] have shown that the vapors escaping from freshly crushed garlic, as well as those from onions are extremely active bactericides. Minchin[4] has presented clinical evidence that garlic and onion have therapeutic value, especially in the treatment of tuberculosis. The present paper deals with the problem of identifying the active agent in garlic. The allyl polysulfides and their relatives, which give garlic its characteristic odor were found by the writers to have negligible bactericidal action. Since aldehydes are known to be present in garlic, it appeared reasonable to try the effect of acrolein (allyl aldehyde). Acrolein was investigated and found to be powerfully bactericidal. All the evidence presented supports the view that acrolein, or possibly some related unsaturated aldehyde is the active bactericide of garlic.

Koch and Fuchs[5] investigated the use of acrolein as an antiseptic, but made no further reference to possible therapeutic application.

There are two possible methods of identifying the bactericidal substance in garlic. One is to separate the complex mixture into its components and to test the bactericidal effects of each. This method appeared more difficult than the following alternative. A series of compounds were prepared on the basis of these clues:

(1) Garlic contains allyl disulfide and related polysulfides. These are the oils giving garlic its characteristic odor.

(2) Garlic and particularly onions contain substances which blister the skin and provoke tears.

(3) The bactericidal substance is volatile, indicating a compound of low molecular weight and simple structure.

The following compounds were synthesized and purified by dis-

* The writers are indebted to Mr. A. K. Humphries, President of the Vitab Products, Inc., San Francisco, for financial assistance.

1 Walker, J. C., Lindegren, C. C., and Bachmann, F. M., *J. Agr. Res.*, 1925, **30**, 175.

2 Walton, L., Herbold, M., and Lindegren, C. C., *Food Res.*, **1**, 163.

3 McKnight, R. S., and Lindegren, C. C., Proc. Soc. Exp. Biol. and Med., 1936, **35**, 477.

4 Minchin, W. C., Bailliere, Tindall, and Cox, London, 1927.

5 Koch, E., and Fuchs, G., *Zentralblatt für Bakteriologie und Parasitenkunde*, 1899, **26**, 560.

tillation at the boiling point characteristic of each: methyl sulfide, ethyl sulfide, ethyl disulfide and allyl disulfide. A mixture of allyl polysulfides up to the pentasulfide was prepared. A portion of this was distilled *in vacuo*. A portion was washed with water in a separatory funnel and used without distillation. The purpose of this was to avoid the possibility of decomposing the higher polysulfides. Allyl isothiocyanate was also prepared.

Alcohols and aldehydes are known to occur in plants and since there are allyl compounds in garlic, it was thought worth while to investigate allyl alcohol and allyl aldehyde (acrolein).

When an agar plate is exposed to the vapors from one gram of freshly crushed garlic for 30 minutes, a sufficient amount of volatile bactericide is taken up by the agar to kill all the bacteria in a heavy suspension of *E. coli* streaked on the surface immediately after exposure. After the plate has been streaked, it is incubated without further exposure to the vapor.

Thirty-minute exposure of a plate to one gram of crushed garlic sterilizes the plate. Furthermore, not more than 10% of any of these compounds could be present in garlic. For these reasons, if 0.1 gm. of a compound does not sterilize the agar surface, it cannot be the active substance in garlic.

Each of the compounds was tested separately, to determine bactericidal effect of direct contact of organism and compound. On the agar surface of one plate was placed 1.0 cc. of the compound. To this was added 0.1 cc. of a heavy suspension of *E. coli* and the mixture was spread over the agar with a glass needle. In a similar manner on a second plate 0.5 cc. of compound and 0.1 cc. of *E. coli* was spread. On a third plate 0.1 cc. of compound and 0.1 cc. of *E. coli* were spread. The plates were then incubated at 37.5°C. for 24 hours. This served to measure the bactericidal activity of the compound in direct contact with the organism.

In a second series, each of the compounds was tested to determine the bactericidal effect of its vapor. A piece of filter paper was placed in a petri-dish cover and moistened with one cc. of the compound. Over this was inverted the petri-dish bottom containing the solidified agar. After exposure for 30 minutes to the vapor of the compound at 37.5°C. the agar was streaked with a heavy suspension of *E. coli*. The cover was then replaced by a clean one and the plate incubated for 24 hours at 37.5°C. The same test was made with 0.5 cc. and 0.1 cc. All the compounds were bactericidal when placed directly on the plates; however, allyl aldehyde was the only compound whose vapors showed an activity comparable with that of the bactericidal substance in garlic.

Bactericidal Activity of Acrolein. Six dilutions of acrolein in water were made ranging from 1:100 to 1:10,000,000. To each member of a duplicate series was added one percent by volume of sterile liquid white of egg (not a solution of dried egg albumin),

TABLE I.
Bactericidal Effects of Acrolein Solutions.

	Albumin absent				Albumin present			
Time, hr.	6	12	24	48	6	12	24	48
E. Coli								
Conc.								
1/100	0	0	0	0	0	0	0	0
1/1,000	0	0	0	0	0	0	0	0
1/10,000	1	0	0	0	1	1	0	0
1/100,000	3	2	0	0	4	4	3	4
1/1,000,000	4	3	2	0	4	4	3	4
1/10,000,000	4	3	2	0	4	4	4	4
Control	4	4	4	4	4	4	4	4
B. Subtilis								
1/100	4	2	0	0	4	4	4	3
1/1,000	4	4	2	4	4	4	4	4
1/10,000	4	4	4	4	4	4	4	4
1/100,000	4	4	4	4	4	4	4	4
1/1,000,000	4	4	4	4	4	4	4	4
1/10,000,000	4	4	4	4	4	4	4	4
Control	4	4	4	4	4	4	4	4

and the mixtures were allowed to stand for 24 hours. Agar plates were exposed for 30 minutes to the vapors from one cc. of each of these solutions placed on paper as described before. These plates were then streaked with *B. subtilis.* A duplicate run was made with *E. coli.* Fresh covers were put on and the dishes were incubated at 37.5°C. for 24 hours. The plates exposed to concentrations of 1:100 were sterile both with or without egg-white. All others showed full or nearly full growth. The vapor pressure of the acrolein above the 1:100 solution was calculated as being 0.6 mm. of mercury. This is approximately one part of gaseous acrolein to 1000 parts of air. The activity of 1 gm. of a 1:100 solution of acrolein is of the same order as the activity of 1 gm. of garlic.

Tests were made to determine the bactericidal activity of aqueous acrolein-solutions upon bacteria immersed in the solution. One-tenth cc. of a heavy suspension of *E. coli* was placed in 10 cc. of each of the serial dilutions. This series was duplicated using a heavy suspension of *B. subtilis.* After 6, 12, 24, and 48 hours, agar plates were streaked and incubated.

The number 4 in Table I indicates growth equivalent to that of the control; zero indicates no growth. The figures 1, 2, and 3 indicate intermediate degrees of growth.

Lewin[6] showed that (1) acrolein is lethal to mammals only in large amounts (0.25 gm. per kilo), (2) acrolein-vapor appears in the breath shortly after subcutaneous injection, (3) the symptoms of acrolein-poisoning in man are dizziness, nausea, and diarrhea. These symptoms have been observed in our laboratory in subjects who have eaten several ounces of garlic. The fact that detectable amounts of acrolein appear in the breath after injection of acrolein suggest its possible value as a disinfectant of the respiratory tract. Since it is lethal only in large amounts and bactericidal in small amounts, we propose to investigate its therapeutic possibilities further.

Summary. The well known sulfides responsible for the peculiar odor of garlic are not responsible for its bactericidal activity. Acrolein was found to be a highly active bactericide. Its properties are such that it gives promise of being a respiratory disinfectant. Its general properties suggest that it or related compounds may be the bactericide of garlic.†

The writers are grateful to Dr. Henry Borsook for his encouraging advice.

9115 P

Acidosis Associated with the Administration of Para-amino-benzene-sulfonamide (Prontylin).

HAMILTON SOUTHWORTH. (Introduced by P. H. Long.) (With the technical assistance of Florence White.)

From the Johns Hopkins Hospital, Baltimore, Md.

In the last 3 months at Johns Hopkins Hospital about 50 cases have been treated with para-amino-benzene-sulfonamide (Prontylin) in doses of 0.04 to 0.12 gm. per kilo per day. Two of these cases have shown clinical acidosis.

Case A. G. J., 29 yrs., colored female, was admitted for an acute beta hemolytic streptococcal tonsillitis. Prontylin was started by mouth and her temperature fell to normal in 36 hours. After 48 hours, however, she began definitely overbreathing and the CO_2 combining power of her plasma was 36.2 vol. %. At this point

[6] Lewin, L., *Arch. Exp. Path. Pharm.*, 1900, **43**, 1351.

† Our recent results show that acrolein is much more poisonous than Lewin found. Proof that acrolein, croton aldehyde, or a similar substance is present in garlic has been obtained by a color reaction.

TABLE I.

Case	Name	Age	Sex	Wt. kg.	CO_2 value before	CO_2 value after	Mean drop	Relation of test to dosage	Aver. dose in gm.	Aver. dose gm./kg.	Aver. dose Days	Aver. dose previous 24 hr.	Total dose gm./kg.	Diagnosis	Organism
1	M.A.	58	F	51	54.1	52.2	1.9	2 days later	3.7	.08	2½	—	.19	Acute tonsillitis, diabetes	Beta strep.
2	A.A.	14	M	49	63.3	44.7	18.6	2 ,, ,,	3.1	.07	3	—	.19	,, ,, peritons. abscess	,, ,,
3	W.M.	21	M	73	58.9	54.1	4.8	1 ,, ,,	3.1	.04	4	—	.18	,, ,,	—
4	M.B.	13	F	34	56.0	49.4	6.6	1 ,, ,,	2.7	.06	5	—	.32	Erysipelas	No beta strep.
5	C.A.	30	F	64	59.8	40.9	18.9	1 ,, ,,	4.1	.06	4	—	.25	Acute rheumatic fever, mitral lesion	Beta strep.
6	L.T.	22	F	75	54.1	48.5	5.6	1 ,, ,,	4.9	.07	2	—	.13	,, tonsillitis and laryngitis	,, ,,
7	W.P.	27	M	69	59.8	52.2	7.6	During dosage	3.7	.05	12	.05	.65	Cellulitis and osteomyelitis of hand	,, ,,
8	R.B.	19	F	45	59.8	40.9	18.9	,, ,,	3.6	.08	4	.08	.32	Strep. pneumonia and otitis media	,, ,,
9	C.K.	17	M	68	59.8	40.9	18.9	,, ,,	4.3	.06	3	.06	.17	Acute otitis, mastoiditis, sinusitis	,, ,,
10	H.F.	28	M	54	57.9	52.2	5.7	,, ,,	3.5	.06	2½	.07	.14	Scarlet fever	Alpha strep.
11	M.D.	20	F	44	63.5	45.7	17.8	,, ,,	5.4	.12	3½	.12	.43	Subac. bact. endocarditis	,, ,,
12	A.G.	31	F	34	63.6	40.9	22.7	,, ,,	5.4	.16	4	.16	.56	,, ,, ,,	,, ,,
13	E.H.	17	F	46	65.5	39.0	26.5	,, ,,	6.0	.12	13+	.12	3.1	,, ,, ,,	,, ,,
14	M.S.	23	F	50	64.5	37.2	27.3	,, ,,	5.4	.11	33	.11	3.5	,, ,, ,,	,, ,,
15	C.S.	30	M	60	54.1	40.0	14.1	,, ,,	5.3	.08	8	.12	.68	,, ,, ,,	,, ,,

Prontylin was stopped (a total of 6.0 gm. or 0.12 gm./kg. had been given) and 8 days later the CO_2 combining power of the plasma had risen to 54.1 vol. %. Two months later, when afebrile and suffering from no apparent streptococcal infection, the patient was readmitted and voluntarily took 6.9 gm. of Prontylin in 60 hours. Again her CO_2 combining power fell, this time from 59.8 to 37.2 vol. %, though there was no definite clinical evidence of acidosis.

Case B. R. E., 45 yrs., white male, had a craniotomy performed with the removal of a dural tumor and 6 days later beta hemolytic streptococci were obtained from the wound. Prontylin was started by mouth and 0.8% solution of para-amino-benzene-sulfonamide was given both subcutaneously and intrathecally, the total dose averaging about 4 gm. or .06-.07 gm./kg. per day. After 6 days he showed marked Kussmaul breathing with a CO_2 combining power of 31.5 vol. %. Later the CO_2 fell to 27.7 vol. %. At a secondary operation the lateral ventricle was entered and the patient died a week later.

Because of these cases a series of 15 consecutive Prontylin-treated patients have been studied for changes in the CO_2 combining power of their blood plasma. In each instance a determination was made prior to the giving of the drug and at least one subsequent one, during or shortly after its administration. The results are given in Table I. Every single case showed some fall in this value. This varied from 1.9 to 27.3 vol. %, and the average decrease for all cases was 14.1 vol. %. In spite of this there was no clinical evidence of acidosis in any case.

It is generally conceded that the CO_2 combining power may be slightly lowered as long as the temperature is elevated, but no specific studies of streptococcal infections are recorded. The effect of fever can be excluded in the first 10 cases, because the preliminary determinations, made during the acute fever, were all normal, and the low values were recorded only in the secondary determinations, all of which were made during convalescence when the temperature was lower, if not normal. It seems unlikely also that the fall was due to any peculiarity inherent in streptococcal infections, because the patients were admitted at all stages of their disease and yet all the preliminary determinations were above 50 vol. %. Moreover, in the second test on Case A, reported above, when she was afebrile and suffering from no streptococcal infection, the CO_2 combining power fell from 59.8 to 37.2 vol. % after the ingestion of 6.9 gm. of Prontylin.

The degree of fall has been found to show a moderate correlation with the dose of Prontylin, in grams per kilogram, given in the

previous 24 hours. Less correlation was found when it was charted against either the average dose or the total dose. However, the fact that in cases 1 and 2 a decrease still persisted when no Prontylin had been given for 2 days shows that the amount given 48 hours previously may still produce an effect.

The mechanism of the acidosis is as yet unknown. Studies to determine its nature are now in progress.

Summary. Two cases of clinical acidosis due to the administration of Prontylin (Para-amino-benzene-sulfonamide) in large doses are reported. Fifteen consecutive cases treated with this drug showed a consistent though variable drop in the CO_2 combining power of their blood plasma.

9116 P

Typhoid Leukocidin.

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Typhoid leukocidin may readily be demonstrated in the filtrate of a 24-hour culture of *Eberthella typhosa* grown in plain sodium chloride veal infusion broth, pH 7.4-7.6, without addition of peptone. Typhoid leukocidin passes readily through Berkefeld N, Chamberland L3, and Seitz EK filters. The leukocidin may be adsorbed by the filter unless suitable precaution is taken.

The demonstration of leukocidal activity may be accomplished by the Neisser-Wechsberg[1] method as used by Gay and Oram,[2] but we have used a method of direct determination which is simple in principle and has yielded more satisfactory quantitative and qualitative data than the older method. For this purpose we have utilized normal rabbit's blood and non-immune human blood. The blood is collected directly into heparin, mixed, and distributed to tubes before there has been any opportunity for the leukocytes to settle out. Equal volumes of varying dilutions of the toxic filtrate are quickly added to the tubes of blood; appropriate control tubes of blood plus plain broth are always included. The tubes are sealed with paraffined corks and incubated at 37°C. in a rotating box for one hour.

1 Neisser and Wechsberg, *Z. f. Hyg. u. Infektionskr.*, 1901, **36**, 299.

2 Gay, F. P., and Oram, F., *J. Immunol.*, 1933, **25**, 501.

Following incubation, the tubes are transferred immediately to a mechanical blood-pipette shaking device and agitation is maintained until total white cell counts and films for differential counts can be obtained. It is necessary to have as many pipettes and counting chambers as there are tubes, to insure a minimal difference of time between the taking of the different counts. Tubes for counting are selected at random rather than in the order of dilution of the filtrate. Counts of control tubes are essential. After the differential counts have been completed, the total number of leukocytes of each type is calculated and the data plotted in order of dilution of the filtrate.

The action of typhoid leukocidin is manifested by a reduction in the number of leukocytes per cmm. of blood-toxin mixture as the concentration of the toxin is increased. When a potent filtrate is used the total count is reduced by about 50%, as compared with the number in the broth-control tube. Furthermore, the neutrophilic granulocyte is the only cell type which is reduced in numbers, and the surviving neutrophils show degenerative changes which are not apparent in the cells from the control tube. By the Neisser-Wechsberg method, leukocytic suspension is inactivated by a concentration of 0.001 cc. of the toxic filtrate. By our method a reduction in total cells and neutrophils is usually definite in 1:1000 dilution of the typhoid filtrate, and in certain experiments destructive activity has been shown by dilutions as great as 1:10,000.

Typhoid leukocidin may be removed from a filtrate by treatment with 3 volumes of 95% alcohol in the cold. The dried precipitate is stable for at least a year without loss in potency. Studies on the effect of heat upon the leukocidal activity of fresh filtrate indicate that potency is reduced 50% at 85°C. for one hour. Further inactivation necessitates holding the toxin at 100°C. for at least 2 hours.

Typhoid leukocidin (filtrate) is completely neutralized by an equal volume of specific concentrated immune globulin (Felix anti-typhoid serum). Increased toxicity for rabbit-neutrophils is apparent when the serum is diluted 1:20, but the neutralizing effect is not lost until the serum has been been diluted to about 1:50,000.

Our data indicate that human neutrophils are somewhat more susceptible to the action of typhoid leukocidin than are the leukocytes of the rabbit. We have found no difference between the leukocidal activity of the Rawlins strain of typhoid bacillus and a typical Vi strain. Filtrates of paratyphoid A and paratyphoid B bacilli also show leukocidal action but the potency is relatively slight.

It is conceivable that typhoid leukocidin may be responsible for the characteristic leukopenia of typhoid fever, the depletion of the myelopoetic elements of the bone marrow, and the absence of in-

flammatory cellular infiltration about foci of typhoid bacilli as seen in sections of tissue obtained postmortem. The postulation of a positive, selective leukocidal action of a soluble toxin *in vivo* is more compatible with the pathology of typhoid fever than is the accepted conception of a negative chemotactic or repellent influence of typhoid bacilli upon granulocytes.

9117

An Improved Optical System for Cathode-Ray Recording.

J. A. Gans. (Introduced by C. J. Wiggers.)

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Recent years have brought the cathode-ray tube into considerable prominence as an instrument for registering and recording electrophysiological phenomena. With the recent advances in high-mu amplifier tubes, the most infinitesimal biological currents come within the scope of cathode-ray study. This has created a need for a reliable and highly efficient optical system for recording on photographic paper. A number of expedients have already been suggested and used, among them those of Gasser and Erlanger,[1] Rijlant,[2] and McCulloch and Wendt.[3]

The methods fall into 2 main divisions: (1) still photography or contact prints of single waves or periodically recurrent phenomena, obtained by photographing a standing wave or the persistent after-image of a single excursion with a still camera, or by holding photosensitive paper in direct contact with the tube screen while the fluorescent dot executes a single excursion across the screen; and (2) photography on paper moving in one axis, of excursions of the dot in a perpendicular axis, this being the method of necessity in the study of continually changing wave forms.

For quite some time the fluorescent screens incorporated in cathode-ray tubes had such low actinic rating and such long persistence of the after-image, that registration was limited to the first type of recording, namely, still photography. However, with the improvement of highly actinic and extremely low persistence screens

1 Gasser, H. S., and Erlanger, J., *Am. J. Physiol.*, 1922, **62**, 496.

2 Rijlant, P., *Gaz. Med. France*, 1934, **4**.

3 McCulloch, W. S., and Wendt, G. R., *Science*, 1936, **83**, 354.

such as the R.C.A. phosphor types 2 and 5, recording on moving bromide paper or film has become a general practice. The various methods in vogue employ a spherical lens to project an exact image of the dark screen and the bright moving dot upon the moving paper. When a round spot of light is so used in recording, the resulting record can in no way compare with the sharp, clean-cut record of hairline recording. However, if the dot can be converted essentially to a hairline, the records must obviously gain in definity and clarity. Such a system has been in use in this laboratory and has been found successful in producing better records. Figure 1 illus-

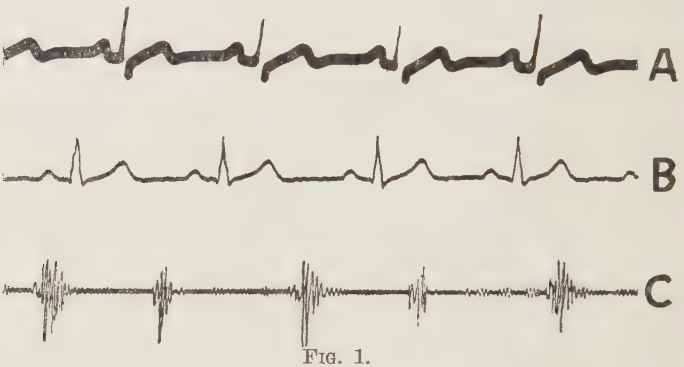

FIG. 1.

trates the effects. A is an electrocardiogram photographed by the round-spot method. B and C are respectively an electrocardiogram and a heart-sound record photographed by the hairline method herein described.

The method consists of substituting crossed cylindrical lenses for the spherical lense of other systems so placed that the image of the round dot becomes essentially a hairline ellipse. While it is difficult to describe such a 3-dimensional system verbally, Fig. 2 will probably convey the idea. The ordinary photokymograph (C) with

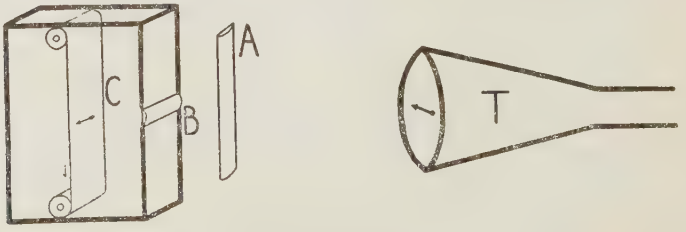

FIG. 2.

its cylindrical lens (B) is used. The cathode-ray tube (T) screen faces the camera, with the dot moving along the axis of lens B.

A second cylindrical lens (A) of slightly longer focal length than lens B is placed between the tube screen and the camera, with its axis crossing that of lens B at a right angle. The position of lens A is adjusted to focus the width of the tracing (analogous to the width of the slit in light-slit recording) and is not critical in adjustment. Lens B is not altered in any way and focusses the fineness of the hairline. Incidentally, the use of the camera with its cylindrical lens possesses the advantage of permitting various other phenomena to be simultaneously recorded by conventional rather than special methods. The number of other phenomena which may be so recorded is limited solely by the width of the photosensitive paper.

So simple is the arrangement and so lacking in critical adjustment that a test tube of the proper diameter filled with clear fluid can be used for lens A with satisfactory results, providing the excursion of the dot is not great enough to introduce spherical aberration.

9118

The Occurrence of Protoporphyrin in the Reticulocytes.

Cecil James Watson and William O. Clarke. (Introduced by J. C. McKinley.)

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Van den Bergh and Hyman[1] described the regular occurrence of protoporphyrin in the erythrocytes. This was proven to be preexistent and not simply derived from hemoglobin during the process of extraction. Subsequently, protoporphyrin was noted in megaloblasts and erythroblasts of the embryonic bone marrow, and of pernicious anemia marrow, by Borst and Königsdörffer.[2] By means of perfusion experiments, Van den Bergh, Grotepass and Revers[3] demonstrated that the surviving liver is capable of converting proto- into coproporphyrin. This led them to suggest that the erythrocyte protoporphyrin might be parent to the coproporphyrin of the

1 Van den Bergh, A. A. H., and Hyman, A. J., *Deutsch. Med. Wchnschr.*, 1928, **54**, 1492.

2 Borst, M., and Königsdörffer, H., Untersuchungen über Porphyrie, S. Hirzel, Leipzig, 223, 1929.

3 Van den Bergh, A. A. H., Grotepass, W., and Revers, F. E., *Klin. Wchnschr.*, 1932, **11**, 1534.

bile, feces, and urine. They evidently assumed that this was coproporphyrin-III, corresponding in configuration to aetioporphyrin-III, hence to hemoglobin. However, coproporphyrin-I has been isolated from normal urine,[4, 10] bile and feces,[5] in increased amounts from the feces of patients with hemolytic jaundice,[6] and pernicious anemia,[7] as well as in most pathological urines having an increased porphyrin content.[4,5,8,9] Coproporphyrin I is not a hemoglobin derivative; the existing evidence[5, 6, 7] indicates that increased amounts of this substance are excreted when there is increased hemopoietic activity. Since the largest amounts have been encountered in hemolytic jaundice, a disease in which active regeneration is constantly manifested by an increase in reticulocytes, the possibility was suggested that the erythrocyte protoporphyrin of Van den Bergh resides in the reticulated cells. The fact noted by Key[11] that reticulocytes are lighter than other erythrocytes, permitted investigation of this question. By centrifuging various samples of blood of different reticulocyte content, upper and lower fractions containing many or few reticulocytes have been obtained; these were subjected individually to Van den Bergh's extraction procedure.[1, 3] The final solutions from each fraction, in equal volumes (2-10 cc.) of 5% HCl, or of ethyl acetate, were directly compared as to intensity of red fluorescence in Wood's light.[1] The light source consisted of a mercury arc lamp.* In this way it was found that the upper fractions regularly yielded a much more intense fluorescence than the lower. This is shown in Table I. The values in the column "amount extracted" indicate the actual fractions of the sample which were employed; thus, in the first instance the upper and lower thirds were extracted while the middle third was discarded. The values given in parentheses represent the amount of packed cells (one-half hour at 3000 r.p.m.) in the fraction. In instances 8-15 inclusive, the measured volume of cells recorded was taken from the top for purpose of comparison with cells from the bottom while the bulk of cells between was discarded. In a number of the experiments, the more fluorescent solution was diluted until the intensity of fluorescence was approximately the same in both solutions. For example, the solu-

4 Watson, C. J., *J. Clin. Investig.*, 1936, **15**, 327.

5 *Ibid.*, in press.

6 *Ibid.*, 1935, **14**, 110.

7 *Ibid.*, 1935, **14**, 116.

8 *Ibid.*, 1935, **14**, 106.

9 Dobriner, K., *J. Biol. Chem.*, 1936, **113**, 1.

10 Hoerburger, W., Inaug. Diss., Erlangen, 1933.

11 Key, J. A., *Arch. Int. Med.*, 1921, **28**, 511.

* Hanovia Analytic Model lamp, with Corning filter No. 587 (3200-4000 A.).

TABLE I.

Source of blood and reticulocytes	% before centrifuging	Amt. extracted after centrifuging		Reticulocyte % after centrifuging		Relative intensity of red fluorescence in final solution	
		Upper	Lower	Upper	Lower	Upper	Lower
1. Pernicious anemia (after peak of retic. response to liver extr.)	5.0	1/3	1/3	9.0	3.8	2	1
2. Famil. hemol. jaundice	8.3	1/3 (3.8cc.)	1/3 (3.8cc.)	14.3	3.0	5	1
3. Normal	1.2	1/4 (15cc.)	1/4 (15cc.)	3.0	0.2	Moderate	Absent
4. Famil. hemol. jaundice	19.5	1/3 (2.1cc.)	1/3 (2.1cc.)	29.0	13.2	3	1
5. " " "	20.0	1/3 (5.2cc.)	1/3 (5.2cc.)	30.0	9.0	Moderate	Faint
6. " " "	16.6	1/3 (3.6cc.)	1/3 (3.6cc.)	22.0	3.5	4	1
7. Hypertension	1.1	1/4 (25cc.)	1/4 (25cc.)	4.1	0.5	Moderate	Absent
8. †Coronary disease	2.0	5.5cc. (200cc.*)	5.5cc.	10.0	0.6	3.5	1
9. † " "	2.0	5.5cc. (45cc.*)	5.5cc.	8.8	0.3	Moderate	Faint
10. †Normal	1.2	5cc. (100cc.*)	5cc.	5.0	0.44	"	"
11. †Polycythemia vera.	1.6	6.1cc. (100cc.*)	8.6cc.	3.8	0.9	"	"
12. Pernicious anemia (after peak of retic. response to liver extr.)	9.0	1.2cc. (12.5cc.*)	1.2cc.	14.0	1.2	"	"
13. Pernicious anemia (after liver therapy)	31.0	2.5cc. (40cc.*)	2.5cc.	68.0	1.0	Intense	"
14. †Pernicious anemia (after liver therapy)	30.0	1.6cc. (38cc.*)	1.6cc.	80-85	1.8	"	"
15. Pernicious anemia (after liver therapy)		2.5cc. (48cc.*)	2.5cc.	8.5	0.7	Moderate	Absent

*Total amount of blood from which respective fractions were obtained.

†In these instances the upper and lower fractions as first obtained were centrifuged again; the amounts noted were then taken from the top of the upper, and bottom of the lower fractions.

tion from the upper fraction in case No. 2 was roughly 5 times as fluorescent as that from the lower fraction. In other of the experiments the intensity of fluorescence is simply described as intense, moderate, faint or absent.

It should be noted that greater red cell concentration (incident to packing) was present in the lower fractions; thus in cases 9 and 10 the amounts noted contained 10,760,000 and 11,200,000 R.B.C. per cmm., respectively, in the upper fractions, but 15,040,000, and 15,400,000 in the lower. The difference in fluorescence between upper and lower fractions was much more marked in instances where the initial reticulocyte counts were high, as in cases of hemolytic jaundice, or of pernicious anemia at the peak of reticulocyte response to liver therapy. The most marked difference in reticulocytes and fluorescence in the two fractions was observed in case 14.

By utilizing the same volume of packed erythrocytes (obtained by centrifuging at 2500 r.p.m. for one-half hour) it was possible to observe a marked increase in erythrocyte-protoporphyrin during the peak of reticulocyte response produced by liver therapy in pernicious anemia, with subsequent marked diminution as the count returned to normal:

a.	11-9-36	Retic.	3.6%	1.4cc.	cells,	no fluorescence
b.	11-11-36	"	38.0%	1.4 "	"	intense fluorescence
c.	11-23-36	"	1.7%	1.4 "	"	very faint fluorescence

The most striking proof of the porphyrin content of the reticulocytes was obtained by administering phenylhydrazine to a rabbit: this was given either subcutaneously or intraperitoneally as follows: 1st day, 0.1 gm.; 3rd day, 0.1 gm.; 5th day, 0.1 gm.; 7th day, 0.2 gm.

On the eighth day the hemoglobin had fallen to 4.1 gm. per 100 cc., the erythrocytes to 1,670,000 per cmm. At this time the reticulocytes numbered 100% of the erythrocytes; after staining with brilliant cresyl blue no red blood cells could be seen, which did not contain at least a moderate amount of reticulated substance: the majority were heavily reticulated. Only occasional nucleated erythrocytes were noted. 0.48 cc. of packed cells from this blood were extracted according to Van den Bergh's method. The red fluorescence of the final 5% HCl solution (8 cc.) was the most intense that has yet been observed in solutions obtained from erythrocytes. The fluorescence of the final solution from 3.8 cc. of packed cells from another rabbit whose blood contained but 7% reticulocytes, was relatively very faint.

Participation of leukocytes or platelets in the production of fluor-

escence in the upper fractions was excluded by a separate extraction of the platelet-leukocyte coat: no fluorescence was demonstrable, although the extract of the underlying, upper strata of red blood cells, containing most of the reticulocytes in the sample, exhibited definite red fluorescence (carried out in case 11). The upper ⅓ (3.6 cc. packed cells) of a blood sample from a patient with pneumonia, who exhibited leukocytosis of 38,000, was extracted without any attempt to remove the buffy coat. The final 5% HCl solution was compared with that from the upper ⅓ (2.1 cc. packed cells) of case 4 (hemolytic jaundice; leukocytes 12,500). In the latter the reticulocytes were 29%, in the former 5%; the fluorescence from the hemolytic jaundice cells was obviously more intense. This constitutes further evidence that the leukocytes are not concerned in the production of fluorescence.

As a result of these findings it is believed that the reticulocytes contain most, if not all, of the protoporphyrin—which Van den Bergh noted in the erythrocytes.† Whether this is in reality the parent substance of the coproporphyrin-I of bile, feces, and urine remains to be determined. Certain findings described in a separate communication[13] support this possibility. A probable lack of strict correlation between reticulocyte percentage and amount of protoporphyrin may account for the failure to identify reticulocytes with "fluorescytes";[12, 14] nevertheless, Müller-Neff[14] has recently concluded that the latter are younger cells, and that an increase in their number indicates increased erythropoietic activity.

Protoporphyrin and brilliant cresyl blue have been found to be mutually precipitable: the addition of a very dilute (almost colorless) solution of crystalline protoporphyrin (from hemin IX), in phosphate buffered physiological saline having a pH of 7.4, to an equal amount of a 0.25% solution of brilliant cresyl blue in physiological saline, is followed by gradual precipitation of a dark blue, almost black substance. At first this appears in a finely divided, nearly imperceptible form; after several minutes the aggregates gradually become larger and sink slowly to the bottom of the tube. This precipitate is not crystalline; microscopically, it is quite similar in appearance to the material seen in reticulocytes stained with

† In the present investigation it was noted that the porphyrin obtained from the red cells has protoporphyrin characteristics, *i. e.*, that it is chloroform soluble and may be extracted from 5% HCl by chloroform; the amounts obtained have not been large enough for spectroscopic identification.

12 Keller and Seggel, *Fol. Haematol.*, 1934, **52**, 241.

13 Seggel, K., *Ibid.*, 1936, **54**, 374.

14 Müller-Neff, H., *Ibid.*, 1936, **56**, 18.

brilliant cresyl blue. Washing the precipitate with physiological saline or water removes but very little of either dye or porphyrin. After solution of the washed precipitate in glacial acetic acid and subsequent mixture with water and ether in a separatory funnel, the protoporphyrin is found in the ether and the brilliant cresyl blue in the aqueous fraction. This behavior, considered with relation to the weak acidic character of protoporphyrin and the basic nature of brilliant cresyl blue, suggests that the precipitate is a salt, and that the acetic acid in dissolving the precipitate, has replaced the porphyrin.

Protoporphyrin behaves similarly toward other basic dyes which have been used in staining reticulocytes, *viz.,* Nile blue sulphate, Janus green B, and methylene blue. Of interest is the observation that the precipitate with methylene blue is much smaller in amount, and appears more slowly; this dye was formerly used in staining reticulocytes, but is much less efficient than brilliant cresyl blue. Wright's stain does not differentiate the reticulated cells. No precipitation occurred in mixtures of this stain and a dilute solution of protoporphyrin. In each of the above instances where a dye was tested with protoporphyrin, control solutions of each substance were observed for the same period; no precipitation occurred. A dilute solution of coproporphyrin (I) has been similarly treated with brilliant cresyl blue without resulting precipitation. These various observations suggest a relationship between protoporphyrin and the supravital staining of the reticulocytes with basic dyes.

Summary. The protoporphyrin which Van den Bergh and Hyman discovered in the red blood cells, has been found to reside chiefly, if not solely, in the reticulocytes. Brilliant cresyl blue and protoporphyrin are mutually precipitable; whether this reaction is responsible for the supravital staining of reticulocytes remains to be determined.

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Response of the Exteriorized Spleen to Ephedrine, Acetyl Choline, Pilocarpine and Pituitrin.

JOHN E. DAVIS. (Introduced by H. B. Haag.)

From the Department of Physiology and Pharmacology, Medical College of Virginia, Richmond.

The importance of the spleen as a blood reservoir has been emphasized by Barcroft,[1, 2] who has calculated that this organ may, by a strong contraction, contribute an additional 10% of erythrocytes to the circulation. Barcroft and Stevens[1] observed, on their "exteriorized spleen" dogs, significant contractions of this organ under various conditions such as excitement, exercise, epinephrine injection, ether administration, etc. By a plethysmographic method, Hunt[3] obtained variable results on the spleen volume of anesthetized cats after the intravenous injection of acetyl choline. Farber[4] invariably noted decrease in volume of the spleen in unconscious dogs following intravenous injection of acetyl choline, and concluded that this drug may act upon the spleen either directly, or indirectly through the splenic nerves. Palitz,[5] using dogs with their spleen surgically placed in a subcutaneous position (but with nerve supply intact), reports splenic contraction following the intravenous injection of pilocarpine. This effect is antagonized by atropine. Simpson, Levy and Cadness[6] report that the subcutaneous injection of ephedrine into normal guinea pigs causes considerable increases of erythrocyte, leukocyte, and thrombocyte numbers, but that these increases are not so great in splenectomized animals. Dale[7] produced diminution of the spleen volume by the intravenous injection of pituitary extract into pithed cats. Sodium amytal anesthesia causes a fall in the erythrocyte number of dogs, which is probably due to relaxation or enlargement of the spleen, according to Essex, Seely, Higgins, and Mann.[8]

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1 Barcroft, J., and Stephens, *J. Phys.*, 1927, **64**, 1.

2 Barcroft, J., *Features in the Architecture of Physiological Function*, Cambridge, 1934.

3 Hunt, Reid, *Am. J. Physiol.*, 1918, **45**, 197, 231.

4 Farber, S., *Arch. Internat. de Pharm. et de Ther.*, 1936, **53**, 367.

5 Palitz, L. L., and Morse, R. P., *Proc. Am. Physiol. Soc.*, 1936, 118.

6 Simpson, Levy S., and Cadness, B. H., *J. Pharm. and Exp. Therap.*, 1936, **56**, 389.

7 Dale, H. H., *Biochem. J.*, 1909, **4**, 434.

8 Essex, H. E., Seely, S. F., Higgins, G. M., and Mann, F. C., PROC. SOC. EXP. BIOL. AND MED., 1936, **35**, 154.

Since anesthetics and trauma may affect the volume of the spleen, and the actions of drugs on that organ, I deemed it worth while to study the influence of several drugs on the spleen size of *fully conscious animals.* Therefore, I used the method of Barcroft and Stevens[1] to exteriorize the spleens of 3 dogs. After recovery from the operation they were trained to lie quietly upon a table. The drugs were injected into the saphenous vein and the length of the spleen was measured by caliper before, and at intervals after the injection.

Table I shows the average extent of shortening of the spleen, of one dog, following drug injections. Generally corresponding results were obtained in the 3 dogs. Contraction occurred practically always within 5 minutes after injection and remained for 25 to 45 minutes. The initial length of this spleen, under basal conditions of the dog and in the absence of excitement, was regularly 3½ inches.

TABLE I.
Average Magnitude of the Splenic Contraction Following Injection of Drugs into the Saphenous Vein of Dog No. 2.

Drug	Dose/Per Kilogram	Shortening in Length of Spleen
Epinephrine HCl	2 cc. 1:100,000/kilo	⅜ inch
Ephedrine Sulphate	1½ mg./kilo	⅜ "
Acetyl choline HCl	0.1 mg./kilo	1/16 "
Pilocarpine HCl	1 mg./kilo	¼ "
Pituitrin (S)	0.3 cc. (total)	⅜ "

Chloroform inhalation to anesthesia was observed (once) to shorten the spleen by ⅜ of an inch. This was only tried once, as was also sodium amytal injection (0.01 gm./kilo) which *elongated* the spleen by ¼ inch.

Barcroft[1] has, of course, observed that epinephrine causes a contraction of the exteriorized spleen, but I have not been able to find any reports of the effects of ephedrine, acetyl choline, pilocarpine or pituitrin on the exteriorized spleen of a conscious dog.

Conclusions. Injection of acetyl choline, pilocarpine, ephedrine, and pituitrin (S) into the saphenous vein causes significant contraction of the exteriorized spleen in conscious dogs. In 2 single observations, chloroform inhalation caused contraction and sodium amytal (intravenously) caused relaxation of the exteriorized spleen.

9120

Failure of Acetylsalicylic Acid to Affect Excretion of Ascorbic Acid (Vitamin C) in Urine.*

JOHN B. YOUMANS, MARVIN B. CORLETTE, HELEN FRANK AND MILDRED CORLETTE. (Introduced by C. S. Robinson.)

From the Department of Medicine, Vanderbilt University School of Medicine.

Daniels and Everson[1] have recently reported that the intake of acetylsalicylic acid increases the urinary excretion of vitamin C in children. Such an action of this drug would be important but to us it seemed unlikely that acetylsalicylic acid had this effect. During a 4-months study of the vitamin C excretion of several subjects kept on a fairly constant intake of the vitamin the drug was taken on several occasions by various subjects without apparent effect on vitamin C excretion.[2] During this time the output of vitamin C remained quite constant, except for intentionally produced variations. Although slight unexplained variations in excretion occasionally occurred, in no instance could they be related to administration of the drug, despite close observation to relate unexplained variations to such factors.

The authors referred to above, state that acetylsalicylic acid "as such or combined with urine" does not react with the 2.6 dichlorophenolindophenol indicator commonly used. It is not clear, however, that they include the conjugation products of salicylates which may be present in the urine and might conceivably give a false reaction for ascorbic acid.

We have studied the effect of ingested acetylsalicylic acid on the excretion of ascorbic acid in 4 subjects. In view of the ease with which ascorbic acid is destroyed by gentle means, such as mild heat or bubbling oxygen through solutions,[2] it was intended to subject the specimens to such procedures with the idea that a failure to reduce the apparent content of vitamin C would indicate that the specimen contained substances which gave a false reaction for the vitamin and were responsible for the apparent increase in the latter. It was realized that failure to reduce the apparent content would not necessarily eliminate the possibility that conjugation products were responsible for the apparent increase in vitamin. It happened, how-

* This study was aided by a grant from the Division of Medical Sciences of the Rockefeller Foundation.

1 Daniels, A. L., and Everson, G. J., PROC. SOC. EXP. BIOL. AND MED., 1936, **35**, 20.

2 Authors' unpublished data.

ever, that this part of the study was unnecessary because no increase in vitamin C excretion in the urine, real or apparent, after the taking of acetylsalicylic acid, was found.

The subjects were 4 young, healthy adults, 2 men and 2 women. They were given a vitamin C-free diet† before and during the ex-

TABLE I.

Influence of Acetylsalicylic Acid on Urinary Excretion of Vitamin C (Ascorbic Acid).

Subject	Date Jan., 1937		Ascorbic Acid—Intake mg.	Ascorbic Acid—Output (urine) mg.
1. J.B.Y.	7		50	27.34
	8		50	27.39
	9		50	28.98
	10		50	22.14
	11	A.a.* 0.6 gm., 9:45 a.m.	50	33.99
	12	A.a.* 1.3 gm., 10 a.m.	50	24.11
	13		50	28.91
	14	A.a.* 1.3 gm., 10 a.m. 1.3 gm., 1:45 p.m.	50	28.48
2. M.B.C.	6		50	39.24
	7		29	29.08
	8		50	27.25
	9		50	28.34
	10		50	37.41
	11		50	23.10
	12	A.a.* 1.3 gm., 10:15 a.m.	38.51	26.65
	13		50	24.12
	14	A.a.* 1.3 gm., 9:45 a.m. 0.6 gm., 12:30 p.m. 0.6 gm., 2 p.m.	50	26.99
3. H.F.	6		50	15.00
	7		50	15.98
	8		50	12.21
	9		50	8.12
	10		50	13.44
	11	A.a.* 0.6 gm., 9 a.m.	50	15.37
	12	A.a.* 1.3 gm., 10:30 a.m.	50	12.05
	13		50	14.14
	14	A.a.* 1.3 gm., 9:20 a.m. 1.3 gm., 1:40 p.m.	50	13.74
4. M.G.C.	6		50	22.98
	7		50	20.02
	8		50	16.35
	9		50	20.78
	10		50	17.31
	11	A.a.* 0.6 gm., 9:30 a.m.	50	16.12
	12	A.a.* 1.3 gm., 10 a.m.	50	18.66
	13		50	20.05
	14	A.a.* 1.3 gm., 9 a.m. 1.3 gm., 1:15 p.m.	50	18.57

*Acetylsalicylic acid.

† The diet consisted of thoroughly cooked meat, cooked eggs and milk, cereals, dried fruit, dried vegetables and butter.

perimental period, to which was added 50 mg. of ascorbic acid daily in the form of orange juice (titrated daily). Twenty-four hour specimens were collected from 8 a. m. to 8 a. m., each individual specimen being examined directly after voiding except in the case of the one or 2 night specimens, which were preserved in 10% of 2N sulphuric acid. After a preliminary period of 5 days to determine the usual daily excretion, acetylsalicylic acid was given in amounts of 0.6 to 2.6 gm. (10 to 40 grains a day), the smaller amounts being taken in a single dose in the morning, the larger in divided doses within about 4 hours. The ascorbic acid content of the urine was determined by the 2.6 dichlorophenolindophenol method as described previously.[3]

The results as shown in Table I indicate that the ingestion of acetylsalicylic acid in amounts as great at 2.6 gm. (40 grains) had no significant effect on the excretion of ascorbic acid in the urine. Neither a significant increase nor decrease occurred in any subject and the slight variations which occurred were no greater than those during the preliminary period. All of the urine specimens following administration of the drug showed a positive test for salicylates (ferric chloride) and occasionally reduced Benedict's solution slightly. It may be added that the excretion of ascorbic acid was of the same order as that observed on other occasions under like conditions, the same persons having served as subjects in previous experiments.

Certain differences between our experiments and those of Daniels and Everson should be noted. Their subjects were children, ours were adults. It is possible that children behave differently from adults in these circumstances, particularly in the presence of fever. Our subjects were afebrile. Their subjects were fed weighed diets and the vitamin C content of the food as well as of the added orange juice was determined. Our diets were not weighed nor the vitamin C content, except that of the orange juice, determined but the nature of our diets was such that an equally constant vitamin intake was obtained and the difference in this respect, if any, should have tended to increase the variation in urinary excretion in our experiments. The vitamin C intake of Daniels and Everson's children varied from about 70 to 102 mg. per day. This is considerably greater in proportion to body weight than our subjects received. Children require more of the vitamin in proportion to weight but our subjects received at least the generally accepted maintenance

[3] Youmans, J. B., Corlette, M. B., Akeroyd, J. H., and Frank, H., *Am. J. Med. Sci.*, 1936, **191**, 319.

requirement for adults, and were in a good state of vitamin C nutrition at the start of the experiment. We believe that these conditions constitute, if anything, a better test of the effect of acetylsalicylic acid on vitamin C excretion. Our experiments indicate that the ingestion of acetylsalicylic acid in daily doses of 0.6 to 2.6 gm. (10 to 40 grains) does not increase (nor decrease) the excretion of vitamin C in the urine in adults.

9121

Ascorbic Acid in Gastric Juice.*

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The literature on vitamin C contains numerous reports on the presence of ascorbic acid in various body fluids and tissues. However, we could find no information on whether vitamin C was present in gastric juice. The fact that normal gastric juice is the most acid of all body secretions suggested that an analysis be made.

First, the gastric juices of dogs were analyzed and later human gastric juice. Nine dogs which had been starved 24 hours were used. Their stomachs were washed with 200 cc. saline and then aspirated. Histamine was given subcutaneously and one hour later the gastric juices were drawn. These fluids were immediately filtered and 25 cc. portions were titrated against the 2-6 dichlorophenolindophenol indicator prepared as described by Bessey and King.[1] The dye was standardized against a standard ascorbic acid (Hoffmann La Roche) solution made up just before analysis, using 25 mg. of the vitamin C per 100 cc. 5% acetic acid. The samples of gastric juice from 9 dogs showed a range of from 0.33 to 1.51 mg. of vitamin C per 100 cc. of gastric juice with an average value of .692 mg.

With a view to ascertaining which part of the gastro-intestinal tract contained the greatest amount of the ascorbic acid, the following analysis was done. The mucosae of the stomach, duodenum,

* Aided by Landon Research Fellowships.

The authors wish to express their appreciation to Dr. H. M. Trusler and Dr. R. N. Harger for their kind assistance.

[1] Bessey, O. A., and King, C. G., *J. Biol. Chem.*, 1933, **103**, 687.

ileum, and colon of dogs No. 2 and No. 9 were analyzed. Using 8% trichloroacetic acid for extraction, the mucosae were scraped off and weighed and then macerated with white sand using a mortar and pestle. After centrifuging and the volume made up to 25 cc. with the trichloracetic acid, the titration was carried out rapidly and in the manner described before, until the first faint pink color was observed. The amount of the dye required for 25 cc. trichloracetic acid was allowed for in all titrations. These results were obtained:

	Mg. ascorbic acid per gram of tissue	
	Dog No. 2	Dog No. 9
Duodenal mucosa	.1095	.2195
Iliac mucosa	.0985	.2073
Colic mucosa	.0605	.1076
Stomach fundic portion	.0526	.0570
Stomach pyloric portion	.00552	.0372

Although the figures on comparison differ considerably, they show separately that the mucosa of the duodenum has the greatest amount of the ascorbic acid, next the ileum, then the colon, gastric fundus, and finally the pylorus.

The next step was to analyze human gastric juice for the vitamin. Twelve hospitalized patients were deprived of their breakfasts, had their stomachs washed and aspirated, and given proper dosages of histamine. The specimens were filtered and analyzed immediately. The preparation of the standard vitamin C solution was the same as described by Farmer and Abt[2] in their analysis for reduced ascorbic acid in blood. The procedure was then carried out in the following way. The strength of the dye was determined in terms of the standard solution of ascorbic acid made up just before each analysis. The gastric juice was dropped into the dye from a 5 cc. micro-burette until the dye was decolorized, *i. e.*, until the dye turned from a pink to a colorless solution.

The samples of human gastric juice from 12 patients showed a range of from .046 to 1.04 mg. vitamin C per 100 cc. of gastric juice with an average value of .397 mg.

We observed on subsequent titrations of dog gastric juice that there was little loss of vitamin C over a 24-hour period, demonstrating the high protective properties of this body fluid, but subsequent analyses on human gastric juice gave different results. Like blood,[3] it appeared from our experiments that the human gastric juice an-

2 Farmer, C. J., and Abt, A. F., Proc. Soc. Exp. Biol. and Med., 1935, **32**, 1625.

3 Pijoan, M., Tounsend, S. R., and Wilson, A., Proc. Soc. Exp. Biol. and Med., 1936, **35**, 224.

alysis should be carried out immediately to get the maximal value for the vitamin C contained in it. It is possible that the explanation of this discrepancy is due to the type of stomach tube employed. Those used on human beings had brass tips, while those used on dogs were rubber catheters. Barron, Barron, and Klemperer[4] have shown that minute amounts of copper destroy the protective power of biological fluids.

Whether the presence of vitamin C in gastric juice has any clinical significance remains to be seen. Work is being carried on to determine any correlation between the amounts of vitamin C in blood and gastric juice.

Conclusions. 1. Gastric juice of dog contains vitamin C in variable amounts. In our series it varied from 0.33 mg. to 1.51 mg. per 100 cc. 2. Rated in descending order according to the amount of vitamin C contained within their mucosae, parts of the gastro-intestinal tract showed the duodenum to have the most, then the ileum, colon, fundus, and finally the pylorus. 3. Human gastric juice also contains variable amounts of vitamin C according to our study ranging from .046 to 1.04 mg. per 100 cc.

9122 P

The Adrenotropic Principle of the Pituitary in Relation to Lactation.*

E. T. GOMEZ AND C. W. TURNER.

From the Department of Dairy Husbandry, Missouri Agricultural Experiment Station.

It was reported[1] that the simultaneous injection of galactin, the adrenal cortical extract (eschatin) and a glucose solution stimulated the initiation and continuation of lactation in hypophysectomized guinea pigs. In the present report the effect of the injection of the adrenotropic hormone of the pituitary as a substitute for eschatin is reported.

The adrenotropic hormone was prepared from whole sheep pit-

[4] Barron, E. S. G., Barron, A. G., and Klemperer, F., *J. Biol. Chem.*, 1936, **116**, 563.

* Contribution from the Department of Dairy Husbandry, Missouri Agricultural Experiment Station, Journal Series No. 497.

[1] Gomez, E. T., and Turner, C. W., PROC. SOC. EXP. BIOL. AND MED., 1936, **35**, 365.

uitaries by a modification of Perla's[2] method.† This extract was found to be free of the growth, gonadotropic and lactogenic factors. In the following discussion this extract will be referred to as A. C. T. (Adreno-Cortico-Tropic). All experimental animals were given 50 mg. of glucose in solution twice daily.

Initiation of lactation. Two unilaterally castrate and experimentally cryptorchid males carrying functional ovarian grafts were given subcutaneous injections of 20 mg. of galactin and 0.1 cc. and 0.2 cc. of A. C. T. simultaneously daily for 3 days beginning immediately after hypophysectomy. Both of these animals were in lactation on the fourth day after the first injection.

Ten multiparous involuted females were prepared for lactation by the injection of theelin‡ (in oil) daily for 20 to 25 days and hypophysectomized 24 hours after the last injection. Immediately after hypophysectomy, one of the animals was given 10 mg. of galactin alone and another 0.2 cc. of A. C. T. daily for 6 days as controls. These animals showed no evidence of lactation.

Five animals receiving 10 to 20 mg. of galactin and 0.1 cc. to 0.2 cc. of A. C. T. were in lactation within 3 to 5 days following hypophysectomy. Two other animals which received 0.1 cc. of A. C. T. and 10 mg. of galactin were in milk 3 days after the first injection. Autopsy of these animals revealed pituitary fragments in their sella turcica. One animal receiving 10 mg. of galactin and 0.1 cc. of A. C. T. showed only evidence of a clear fluid secretion after 6 days. In addition, one non-hypophysectomized control receiving 10 mg. of galactin was in lactation after 2 days, while another receiving 0.2 cc. of A. C. T. showed no evidence of lactation after 6 days.

Maintenance of lactation. Eight lactating guinea pigs hypophysectomized 2 to 7 days after parturition were immediately injected with 10 to 20 mg. of galactin and 0.1 to 0.2 cc. of A. C. T. daily. These animals were maintained in lactation for periods ranging from 8 to 15 days. Two animals receiving 10 mg. of galactin and 0.5 cc. and 0.25 cc. of A. C. T. succumbed after 3 and 5 days respectively. The mammary glands of these animals showed abundant milk secretion at autopsy. Two other animals receiving 10 and 20 mg. of galactin and 0.1 cc. and 0.2 cc. of A. C. T. succumbed after 4 and 9 days. Autopsy of these animals revealed pituitary fragments in the sella

2 Perla, D., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 751.

† Details of the method of extraction and assay to be published later. One cc. of the extract is equivalent to 100 mg. dry whole pituitary powder.

‡ Kindly supplied by Dr. Oliver Kamm of Parke, Davis and Co.

turcica. In addition, one hypophysectomized lactating guinea pig receiving 10 mg. of galactin and another 0.5 cc. of A. C. T. ceased lactating 2 days after the operation. Lactation was reinitiated in these animals following 3 daily injections of 10 mg. of galactin and 0.2 cc. of A. C. T. beginning immediately after the complete cessation of milk secretion.

Summary. These observations are believed to indicate that the reason for the cessation of lactation following hypophysectomy in the guinea pig is the withdrawal of the lactogenic, the adrenotropic, and probably the carbohydrate metabolism hormones of the pituitary.

9123 P

Effect of Thyroxine and Galactin on Lactation in Hypophysectomized Guinea Pigs.*

E. T. GOMEZ AND C. W. TURNER.

From the Department of Dairy Husbandry, Missouri Agricultural Experiment Station.

Following our observations[1] that the relatively pure lactogenic hormone (galactin) was incapable of initiating or maintaining lactation in hypophysectomized guinea pigs, experiments were initiated to determine what other pituitary principles played a direct or indirect rôle in lactation. As it is well established that the adrenal (cortex) and thyroid glands atrophy following hypophysectomy, our attention was turned to the hormones of these glands.

In this communication the effect of the sodium salt of pure thyroxine† alone and in combination with galactin on lactation in hypophysectomized guinea pigs is reported. To prevent the cessation of lactation attributable to the shock of the operation, all animals (unless otherwise stated) were given 5 to 10 mg. of whole sheep pituitary (powder) daily for 2 days. In addition, throughout all the experiments 50 mg. of glucose in solution were given twice daily.

Two hypophysectomized lactating guinea pigs were given 0.05

* Contribution from the Department of Dairy Husbandry, Missouri Agricultural Experiment Station, Journal Series No. 498.

1 Gomez, E. T., and Turner, C. W., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 404.

† Prepared by the British Drug House, Ltd., London.

and 0.1 mg. of thyroxine daily beginning on the third day after hypophysectomy. Both of these animals ceased lactating after 2 days. Another animal receiving 0.05 mg. of thyroxine daily showed evidence of milk secretion for 5 days. At autopsy the mammary glands of this animal showed slight milk secretion. Microscopic examination of the sectioned sella revealed the presence of anterior pituitary fragments and the whole posterior lobe.

Two normal lactating females and one experimentally induced lactating male were given 5 and 10 mg. of galactin and 0.025 and 0.05 mg. of thyroxine daily immediately following hypophysectomy. The rapid cessation of lactation which follows hypophysectomy was not prevented in these animals, the mammary glands being dry in 2 to 3 days.

Six lactating guinea pigs were given 0.025 to 0.1 mg. of thyroxine and 5 to 10 mg. of galactin beginning on the third day following hypophysectomy. Three of these animals which received 0.025 and 0.05 mg. of thyroxine and 5 and 10 mg. of galactin showed no evidence of milk secretion after 3 days. Lactation was reinitiated in these animals following 2 to 3 daily injections of 5 to 10 mg. of galactin and 0.2 to 0.4 dog units of eschatin‡ (adrenal-cortical extract). However, when thyroxine was substituted for eschatin, milk secretion decreased rapidly, the glands being dry in 2 to 4 days. Two other animals receiving 0.07 and 0.1 mg. of thyroxine and 10 mg. of galactin showed no evidence of milk secretion after 2 days. Both of these animals developed severe coma after 2 injections. However, they were resuscitated by the injection of 100 mg. of glucose in solution administered 4 times daily. The sixth animal receiving 5 mg. of galactin and 0.05 mg. of thyroxine showed slight milk secretion for 5 days. Microscopic examination of the sella revealed the presence of pituitary fragments.

Summary. These observations are believed to indicate that the atrophy of the thyroid glands and the probable decrease in the secretion of thyroxine is not a limiting factor in the rapid cessation of lactation observed in the hypophysectomized guinea pig. This conclusion should not be interpreted as indicating the lack of importance of the thyroid in lactation, as it has been shown by Graham[2] and others that the milk yield of dairy cattle may be reduced by thyroidectomy or increased by administration of thyroxine.

‡ Kindly supplied by Dr. Oliver Kamm of Parke, Davis and Company.

2 Graham, W. R., Jr., *Biochem. J.*, 1934, **28**, 1368.

9124 P

Effects of Prolonged Chronic Vitamin A Deficiency in the Rat With Special Reference to Odontomas.*

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The gross and microscopic pathological changes induced by a relatively acute vitamin A deficiency have been thoroughly studied[1] and the mechanism of the process of repair described.[2, 3] On the other hand, a mild chronic deficiency of the vitamin, such as is more likely to occur in man, has received less attention. The production of such a borderline condition in animals is attended by many difficulties; chief among these is the fact that an excess of the vitamin is readily stored and external evidence of the deficiency removed whereas too small a supply results in the more acute type of the deficiency and in death. The following procedure, however, was found satisfactory for the production of a chronic deficiency of long duration.

Albino rats were specially prepared by withdrawing vitamin A from the mother's diet before the young were 13 days old. At weaning (21 days) the animals were caged individually and fed a purified, vitamin A-free ration adequate in all other respects. When either beginning decline in weight, the first stages of xerophthalmia, or continued excess cornification of the vaginal epithelium of the females appeared (at an average age of 52 days), a small but known quantity of vitamin A in the form of cod liver oil† was administered by mouth. Thereafter, the amount of vitamin A to be given each day was determined for each individual rat from its clinical state (body weight, appearance of eyes, snuffles, and vaginal smear). Thus, the quantity of the vitamin was so adjusted that the animals were maintained in a state of incipient vitamin A deficiency for periods up to one year. The average daily dose of vitamin A required varied from 0.7 to 6.0 (range 0.7-8.0) Inter-

* A contribution from the Dental Study Group, Yale University School of Medicine.

1 See current review, Robertson, E. C., *Am. J. Med. Sci.*, 1936, **192**, 409.

2 Wolbach, S. B., and Howe, P. R., *J. Exp. Med.*, 1933, **57**, 511.

3 Wolbach, S. B., and Howe, P. R., *Am. J. Path.*, 1933, **9**, 275.

† The cod liver oil was assayed in our laboratory by the method prescribed in the U. S. Pharmacopeia XI.

national Units. A general relationship existed between the dose required and the age and/or weight of the animals; the dose had to be increased progressively throughout the experiment.

The averaged growth curves were normal in shape, but the absolute values were slightly low. There was a 54% mortality among animals between the ages of 81 and 365 days, the majority of deaths resulting from tracheal obstruction by a muco-purulent plug. Otitis media, sinusitis, and congested nares were commonly observed. Large numbers of calculi were found frequently in the urinary bladder both in the animals that died earlier and in those that lived for a year.

The most striking and consistent effect of the chronic deficiency was on the incisor teeth, beginning when the rats were about 50 days of age. These teeth showed progressively a loss of the normal orange pigment, the development of opacity, a distortion of shape (crossing, twisting, transverse and longitudinal ridging), and eventual exfoliation of the erupted portion. Large solid lumps, not sensitive to the touch, developed in the maxillae.

The incisor teeth showed histological changes similar to those observed by Wolbach and Howe[8] in rats fed a diet free of vitamin A. The majority of animals surviving 365 days on the low vitamin A diet developed, in addition, tumor growths (odontomata) and, in a few instances, supernumerary incisor teeth. The tumors arose from the pulp and consisted chiefly of spindle-shaped cells similar to the embryonic cells of the pulp tissue. Some of the tumors proliferated to the point of replacement of the entire alveolar bony structures of both the upper and lower jaws and even extended to and ulcerated through the gingival margin. Small groups of squamous epithelial cells were distributed throughout the spindle cell proliferations, some of which had undergone complete keratinization. Groups of columnar cells resembling odontoblasts also occurred. Many of these aggregates of cells were adjacent to atypical dentine and osteodentinal structures. Imperfect forms of germinal tooth bud structures were found in some of the tumors. The original incisor teeth of the majority of the rats were greatly distorted or replaced by the tumor growth. The ameloblasts of the enamel organs of all of the teeth developing odontomas were atrophied and metaplasia to squamous epithelium was encountered. Tumor proliferations were not present in the molar teeth of these rats, possibly because these teeth are not of the continuously-growing type, as are the incisors, and probably because they were fully developed before the deficiency became manifest. It is possible that the inevitable repeated slight decline and recovery of the animals,

incident to the method of feeding, have emphasized the production of denticles in the incisors which has been noted by Wolbach and Howe[8] in the more acute condition.

9125 P

Nature of the Anticlotting Activity of Streptococci *in vitro*.

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While studying factors affecting the formation of the fibrinous inflammatory barrier in acute local streptococcal inflammation, Dennis and Berberian[1] confirmed the observations of Tillett and Garner[2] on streptococcal fibrinolysin, and described the presence of an anticoagulant in filtrates of certain strains of hemolytic streptococci and of virulent viridans streptococci. Although certain strains of erysipelas streptococci produced both fibrinolytic and anticoagulant factors, sound evidence was offered that we were dealing with 2 distinctly different substances. The differences were: fibrinolysin was never produced by *Strep. viridans;* fibrinolysin acted only upon human plasma clot, while the anticoagulant prevented the clotting of both human and rabbit recalcified oxalated plasma; and fibrinolysin was thermolabile while the anticoagulant was thermostable. Anticlotting factor masked the action of fibrinolysin whenever the two occurred together. Tunnicliff[3] confirmed our observations and correlated the anticlotting activity with the smooth (S) phase of greening streptococci. Neter and Witebsky,[4] apparently unaware of our earlier observations, recently reported on the anticoagulant activity of streptococcal filtrates, but failed to recognize the fundamental difference between anticoagulant and fibrinolytic phenomena.

During the past 3 years we have extended our observations on

* Observations on purification of the anticlotting were made by one of us (E. W. D.) during tenure of a Rockefeller Research Fellowship in Bacteriology, at Harvard Medical School, 1934-35.

1 Dennis, E. W., and Berberian, D. A., *J. Exp. Med.*, 1934, **60**, 581.

2 Tillett, W. S., and Garner, R. L., *ibid.*, 1933, **58**, 485.

3 Tunnicliff, R., *J. Infect. Diseases*, 1936, **58**, 92.

4 Neter, E., and Witebsky, E., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 549, 858.

the nature of the anticlotting activity, using 45 strains of *Strep. hemolyticus* and 18 strains of *Strep. viridans*. We have used the original method of Tillett and Garner[2] throughout for the demonstration of fibrinolytic and anticlotting action.

Using filtrate of an E_1 strain of *S. hemolyticus* which was both fibrinolytic and anticoagulant, and various *S. viridans* filtrates which were anticoagulant only, an effort was made to isolate and identify the anticlotting factor. It was found that the anticoagulant is soluble in 75% alcohol, absolute alcohol, and ether. It is readily dialyzable when in a relatively pure state, and gives a strongly positive Kelling's test for lactic acid. We have concluded that the anticoagulant factor of streptococcal cultures is primarily lactic acid, perhaps with a minor admixture of other organic acids.

Having determined that it is not the lactate ion which is responsible for inhibition of coagulation, the relationship between anticlotting action and acid content of streptococcal filtrate was then carefully studied. Manifestation of anticlotting action is dependent upon (a) the amount of fermentable sugar in the broth, and (b) the degree of buffering of the medium. Anticlotting action was seldom shown if the medium contained less than 0.4% dextrose. Observations on the relative importance of pH of the filtrate and total acid content showed that the anticlotting activity is correlated with the total acid rather than any particular pH; the acidity of the plasma-clot test mixture is the determining factor.

We were not able to establish definite relationship between anticlotting activity and the dissociative phase of the organism, since certain apparently rough strains inhibited coagulation. However, rapid subculturing, with increased "smoothness" of the culture, was always paralleled by increased acid content and increased anticlotting activity of the filtrate.

The recent report by Dart[5] on the solubility of the anticoagulant in alcohol, and on its thermostability, is entirely in harmony with our earlier observations and the results reported above.

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[5] Dart, E. E., *ibid.*, 1936, **35**, 285.

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The Establishment of Certain Reflex Arcs in Foetal Sheep.

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Sheep embryos first respond to mechanical and electrical stimuli applied to them between the 33rd and 35th day after insemination. At this time movements may be elicited by tapping on the unopened amniotic sac or by faradic currents applied to the spinal cord or to the face. The movements appear to be due to the localized contraction of the muscles in the neck, the fore or the hind limbs. The actual neural elements concerned in the production of the movements of the limbs are not definitely known, but it is clear that these movements themselves are not as yet a part of a total mass reaction.[1]

If the movements of the limbs in these embryos could be shown to involve at least a primary sensory neuron and a motor neuron, the observations would lend support to the general thesis that behavior has its genesis in individual reflexes which are later associated into reaction patterns. We have, therefore, sought to produce isolated movements of the type observed in the sheep embryos under conditions in which we were certain a primary afferent neuron was stimulated as the first element in a reflex arc.

Mechanical stimuli such as stroking the forelimb with a glass rod or flipping it do not induce either flexion or extension movements in sheep fetuses until 44-47 days after insemination. Reflexes in the hind legs cannot be elicited in these ways until 3 or 4 days later. There remains in these cases the possibility that the stimuli were not adequate in younger embryos to produce reflexes in the limbs.

Fortunately fetuses 42 days old and slightly younger are large enough to be manipulated and dissected. We have found it possible to dissect out and to section the median nerve and also the lateral popliteal. Faradic currents could then be applied to their central ends. No movements in the ipsilateral limb or any other part of the body have ever followed faradization of the central end of the median nerve in fetuses younger than 44 days old, though movements can always be produced in older fetuses under similar conditions. Likewise no movements have been produced in the ipsilateral hind leg when the central end of the lateral popliteal nerve was stimulated in fetuses younger than 47 days. In fetuses 47

[1] Barcroft, J., Barron, D. H., and Windle, W. F., *J. Phys.*, 1936, **87**, 73.

days old and over, movements of the ipsilateral leg are quite readily obtained when the central end of the lateral popliteal nerve is stimulated with currents of moderate strength.

However, faradization of the peripheral end of either the median nerve or the lateral popliteal nerve produced muscle contraction with currents of the same strength as those applied to the central ends in each of the fetuses.

These observations suggest that prior to this time (44 days for the fore leg and 47 days for the hind) the primary afferent neurons distributed to the limbs have not made functional connections with the anterior horn cells supplying the limb musculature. Histological studies of the neurofibrillar development of the spinal cords of a series of sheep embryos demonstrate, however, that sensory collaterals reach the gray matter as early as the 32nd day after insemination. The first appearance of collaterals in the gray matter, therefore, does not appear to be directly related to the time at which reflexes may be elicited. Further, it would appear that the stimuli inducing the movements seen in sheep embryos 33 to 35 days old did not excite the anterior horn cells *via* the primary afferent neurons distributed to the limbs. These movements cannot, therefore, be regarded as true reflexes. The implication is that behavior does not appear first in the form of isolated reflexes which are later collected and organized into reaction patterns.